N° d'ordre…

الجمهورية الجزائرية الديمقراطية الشعبية

PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

DJILLALI LIABES UNIVERSITY OF SIDI BEL ABBES

FACULY OF NATURE AND LIFE SCIENCES
Department of Environment Sciences
Research Laboratory: '*Biodiversité Végétale: Conservation et Valorisation*'

THESIS

Submitted for the degree of

DOCTORATE IN SCIENCES

Speciality : Environment Sciences
Option : Applied Ecology

Presented by :

Asma EL ZEREY-BELASKRI born Asma BELASKRI

A MULTIDISCIPLINARY APPROACH FOR THE CHARACTERIZATION OF *Pistacia atlantica* Desf. subsp. *atlantica* DIVERSITY IN NORTHWEST ALGERIA

Defended on

In front of the honourable committee members:

Chairman	**Pr Fawzia ATIK-BEKKARA**	**Univ. Tlemcen (Algeria)**
Examiner	**Dr Hassiba BESSAM**	**Univ. Sidi Bel Abbes (Algeria)**
Examiner	**Pr Ahmed OUHAMMOU**	**Univ. Marrakech (Morocco)**
Examiner	**Pr Abderrahmane ROMANE**	**Univ. Marrakech (Morocco)**
Supervisor	**Pr. Hachemi BENHASSAINI**	**Univ. Sidi Bel Abbes (Algeria)**
Invited member	**Pr. Ligia SALGUEIRO**	**Univ. Coimbra (Portugal)**

Academic year 2015 – 2016

N° d'ordre…

الجمهورية الجزائرية الديمقراطية الشعبية

PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

DJILLALI LIABES UNIVERSITY OF SIDI BEL ABBES

FACULY OF NATURE AND LIFE SCIENCES
Department of Environment Sciences
Research Laboratory: '*Biodiversité Végétale: Conservation et Valorisation*'

THESIS

Submitted for the degree of

DOCTORATE IN SCIENCES

Speciality : Environment Sciences
Option : Applied Ecology

Presented by :

Asma EL ZEREY-BELASKRI born Asma BELASKRI

A MULTIDISCIPLINARY APPROACH FOR THE CHARACTERIZATION OF *Pistacia atlantica* Desf. subsp. *atlantica* DIVERSITY IN NORTHWEST ALGERIA

Defended on

In front of the honourable committee members:

Chairman	**Pr Fawzia ATIK-BEKKARA**	**Univ. Tlemcen (Algeria)**
Examiner	**Dr Hassiba BESSAM**	**Univ. Sidi Bel Abbes (Algeria)**
Examiner	**Pr Ahmed OUHAMMOU**	**Univ. Marrakech (Morocco)**
Examiner	**Pr Abderrahmane ROMANE**	**Univ. Marrakech (Morocco)**
Supervisor	**Pr. Hachemi BENHASSAINI**	**Univ. Sidi Bel Abbes (Algeria)**
Invited member	**Pr. Ligia SALGUEIRO**	**Univ. Coimbra (Portugal)**

Academic year 2015 – 2016

بسم الله الرحمن الرحيم

سورة العنكبوت

20

DEDICACE

A.P.J. Abdul Kalam [1931-2005] a dit un jour:

"**L**es rêves ne sont pas ce que tu vois dans ton sommeil. Les rêves sont ces choses qui t'empêchent de dormir."

C'était mon cas avec la variabilité chez *Pistacia atlantica. Heureusement, y'avaient ceux qui ont cru en moi et en mon rêve. Derriere mon rêve y'avait aussi le* ***leur****.*

Ni ma volonté, ni ma détermination, ni les chers produits et financements que j'ai consommés n'auraient permis à ce que j'aboutisse aujourd'hui à cette fin sans les ***prières*** *de cette femme qui, sans cesse,* ***a sacrifié*** *pour moi. Si toutes les mères le font,*

Ma mère l'a fait et continue de le faire très originalement

Je n'aurai jamais pu faire autant de sorties sur terrain, autant d'échantillonnages, autant de répétitions, autant de déceptions, et autant de ***succès****, s'il n'était pas avec moi tantôt pour alimenter mon enthousiasme et tant d'autre pour m'encourager quand l'épuisement et la fatigue me gagnaient*

.

Mon père l'Unique par excellence,

Je vous aime, je vous dédie cette thèse, et je prie Allah de vous récompenser pour tout ce que j'ai appris et pour tout ce que je transmettrai.

Asma

ACKNOLEDGMENTS

First of all, obviously this multidisciplinary study would never have been possible without the effective collaboration of several laboratories in various scientific fields and many people who were available when I needed them. I would like to acknowledge them by expressing sincere thanks hopefully my worlds will achieve their hearts.

At the end of this work, I would like to extend my deepest gratitude to someone who direct me to the way of knowledge almost fourteen years ago, who was the first source of inspiration. I have gained the wealth of knowledge, experience and skills in botanics and taxonomy from him before starting this thesis. Throughout this work, I appreciated his guidance, scientific support and I am very grateful for the chance he gave me to work with him and learn from him. Twice my supervisor, he was the quintessential instructor, untiring and available. To Pr Hachemi BENHASSAINI, my supervisor, all the acknowledgments are addressed.

I would also like to extend my words of appreciation to the committee members. I had the opportunity to know them before and I believe that, there were different circumstances I met them, but I was the luckiest and the most honored. So,

I owe my gratitude to the committee president, Pr Fawzia Atik-Bekkara from the Laboratory (Produits Naturelles, Equipe: Etude des composés volatils (huiles essentielles) et des composés phénoliques (flavonoïdes), University of Tlemcen). I do believe that all her advice, commitments and comments are for the benefit.

I am deeply grateful to the examiners Dr Hassiba Bessam, Department of Biologie, Faculty of Nature and life sciences (University of Sidi Bel Abbes), Pr Ahmed Ouhammou (Laboratoire Ecologie et Environnement, Faculté des Sciences Semlalia, Université Cadi Ayyad, Marrakech, Maroc), and Pr Abderrahmane Romane (Laboratoire de chimie organique appliquée, Département de Chimie, Faculté des Sciences (Semlalia), Université Cadi Ayyad, Marrakech, Maroc). I am grateful to them, I could learn as much by their criticism and their advices and experiences. Beside, their encouragements have never failed. Most of all, they paid me great honor by accepting to join the committee.

As Invited member, Pr Ligia Salgueiro is warmly appreciated for the interest she always showed for my work and for honoring my thesis defense. I am grateful for all what I learned from her and from her team in the laboratory of Pharmacognosy, University of Coimbra, Portugal.

I am grateful to the Ministry of Higher Education and Scientific Research (Algeria), Djillali Liabes University (Sidi Bel Abbes), Faculty of Nature and Life Sciences for the awarded scholarship (N° de la décision 50/FSNV/VDPG/SCD/2012; N° de la décision 85/FSNV/VDPG/SCD/2013; N° de la décision 136/FSNV/VDPG/SCD/2014) which supported a great part of this thesis and enabled me to accomplish my project.

I am grateful to the project FCT, Portugal (PEst-OE/SAU/UI0177/2011) and to the project SMEC - ERDF-European Union - Spain (AGL2013-43732-R) for the technical and financial support of parts of my thesis.

I am very grateful to all who provided help and technical support at the foreign laboratories (the Laboratory of Pharmacognosy and the Centre for Functional Ecology, University of Coimbra, Portugal; the Laboratory of Cytogenomic, DRAT, Lisbon, Portugal; and the Institute for Mediterranean and Subtropical Horticulture, University of Malaga, Spain).

I believe that he (Dr Wael ABED EL ZEREY) came to Algeria just for me: to meet me, to help me, to support me and to map my thesis sampling area, but most of all, to map for me happiness and love. For you, best friend I ever get, gratitude and love.

المجد والخلود للشهداء
Thank you Algeria
Eternal glory to our martyrs

Asma

ملخص منهجية متعددة التخصصات لتشخيص التنوع الحيوي لشجرة البطم الاطلسي في شمال غرب الجزائر

تهدف الدراسة الحالية إلى توصيف تنوع البطم الأطلسي في شمال غرب الجزائر. تم تشخيص ستة عشر شعبة طبيعية من البطم الأطلسي على التدرج الطولي. البيانات الخاصة بدرجة الحرارة و التساقطات تم استخدامها من مواقع أخذ العينات للتوصيف المناخي. تناولت الدراسة النمط الظاهري لعشر أشجار في كل موقع و التباين الشكلي و القياسي ل 3520 ورقة مركبة كاملة النمو. قدمت الدراسة الحالية بيانات قياسية جديدة، حيث يصل طول الورقة إلى 24.5 سم و عرض الورقة 21.9 سم ، و طول سويق الوريقة النهائية الى 3.4 سم ، سجل عدد يصل إلى 18 وريقة للورقة المركبة . استغلت نتائج هذه الدراسة لتحديث مفتاح هوية البطم الاطلسي فرع الاطلسي. تم تقييم التباين الكيميائي من خلال تشخيص الزيوت الأساسية المستخلصة من الأوراق والسكريات المتعددة الجدارية. استخلصت الزيوت بواسطة التقطير المائي ، ثم حللت عن طريق الاستثراب الغازي ، ثم الاستثراب الغازي الموصول بمطياف الكتلة تراوح عائد الزيوت بين 0.1 بالمئة و 0.42 بالمئة . سجلت أربع مكونات غالبة ، حيث لم يتم ذكر أخر مكونين كمكونات غالبة للزيوت الأساسية للبطم الاطلسي. يصنف التحليل العنقودي متعدد المتغيرات الزيوت الأساسية إلى ثلاثة أنماط حسب المكونات الغالبة ، تم استخلاص و تقييم السكريات الجدارية (السيليلوز و الايميسليلوز) من الأوراق مكتملة النمو و تراوح عائد المستخلص الجداري بين 36.2 و 96.5 بالمئة بينما تراوح عائد السليلوز و الايميسليلوز بين 21.25 بالمئة و 51 بالمئة و 7 بالمئة و 27.6 بالمئة على التوالي. تم تقييم التنوع الوراثي من خلال تقدير حجم المحتوى الوراثي(المجموع الوراثي) ، تعداد الصبغيات و تقييم التنوع الجيني. قدر حجم المحتوى الوراثي باستخدام تقنية قياس التدفق الخلوي على 97 عينة ،قيمت الإحصاءات الوصفية المتوسط ، الانحراف المعياري و معامل الاختلاف المتوسط في كل موقع. أثبتت كثافة الوميض الفلوريسني لايوداید البروبديوم أن جميع العينات ثنائية الصيغة (2ن = 2س) و أن البطم الاطلسي يتميز بمحتوى وراثي ذو حجم صغير جدا (1.21 ± 0.02بيكوجرام) لم تسجل في التحليل الإحصائي فروق ذات دلالة إحصائية في حجم المحتوى الوراثي بين العينات المدروسة. تم دراسة المورثات و تعدادها بطريقة الدق (سكواش) على براعم زهور ذكرية حيث أعطت أفضل النتائج و سمحت بتعداد 15 زوج مورثة = 30 مورثة في الطور قبل الاستوائي للانقسام المتساوي. أما بالنسبة لدراسة التنوع الجيني شخصت 61 عينة من مختلف المواقع بواسطة 6 توابع دقيقة . تم تضاعف 26 أليلة و تراوح التعداد الفعال الاليلي بين 1.92 و 4.34. تراوحت ترددات الاليل بين 0.008 و 0.68 حيث لوحظت 8 اليلات نادرة. لم يثبت اختلاف في توازن هاردي وانبغ ، وصنفت أربع توابع على نجاعتها. اظهر التحليل الجزيئي للتباين تمايز معتدل في ترددات الاليل بين أربع مجموعات التي صنفت على أساس الموقع الجغرافي . اظهر التحليل UPGMA أن الاختلاف الجيني يلاحظ داخل الموقع و بين المواقع. أثبتت الدراسة الحالية وجود تنوع وراثي داخل الفرع الاطلسي، من المرجح انه يرجع للتدفق الجيني بين الأنماط و بين التجمعات الاحيائية الطبيعية لشجرة البطم الاطلسي.

الكلمات الاساسية : البطم الأطلسي. شمال غرب الجزائر. التنوع الظاهري. تشخيص المناخ. مفتاح الهوية؛ الزيوت الأساسية ؛ السكريات الجدارية . المجموع الوراثي. قياس التدفق الخلوي. تعداد الصبغيات. الاختلاف الوراثي .التوابع الدقيقة.

Abstract **A MULTIDISCIPLINARY APPROACH FOR THE CHARACTERIZATION OF *Pistacia atlantica* Desf. subsp *atlantica* DIVERSITY IN NORTHWEST ALGERIA**

The current study aims at the characterization of *Pistacia atlantica* Desf. subsp *atlantica* diversity from Northwest Algeria

Sixteen natural populations of *Pistacia atlantica* Desf. subsp. *atlantica* were investigated on a longitudinal gradient. The pluviothermal data of sampling sites were used for the climatic characterization. The study examined morphologically ten mature trees per sites (males and females) and biometrically 3520 mature compound leaves. A significant correlation between climatic parameters and leaf morphology was showed. Several values were reported for the first time on the species, such as the length and the width of the leaf (reaching up to 24.5 cm/21.9 cm), the leaflets number (up to 18 leaflets/ leaf) and the petiole length of the terminal leaflet (reaching up to 3.4 cm). The original findings of this study were used to update the *Pistacia atlantica* subsp. *atlantica* identification key. The intraspecific chemical variability was assessed through the characterization of leaf essential oils and cell wall polysaccharides. Essential oils were isolated by hydrodistillation. Compositions were further investigated by Gas Chromatography (GC) followed by Gas chromatography-mass spectrometry GC/MS and were submitted to multivariate statistical analysis. Essential oils were obtained in yields ranging from 0.1% to 0.42%. Terpinene-4-ol, α-pinene, germacrene D and *E*-caryophyllene were found as major components. The two last compounds were never mentioned as main constituents in the essential oil of this species. Multivariate cluster analysis of compositional data evidenced three types of essential oil considering the predominant constituents: 1) terpinene-4-ol ; 2) α-pinene / camphene ; 3) α-pinene / germacrene D. For the cell wall polysaccharides dosage, cellulosic and hemicellulosic fractions were isolated and dosed from mature leaves. The wall residue yield varied from 36.2 % to 69.5%. The cellulose and hemicelluloses yields varied from 21.25% to 51% and 7% to 27.6% respectively. The genetic diversity was assessed through the genome size estimation, the chromosome count and the genetic polymorphism assessment. The genome size estimation was performed using flow cytometry on 97 samples. The Propidium Iodide (IP) fluorescence intensity showed that all the analyzed samples are diploids (2*n*=2x). The flow cytometry analysis leaded to estimate a very small genome (2C = 1.21±0.02 pg). No statistical differences were recorded between the genome sizes of the analyzed samples. Squash preparation for chromosome study was carried on fixed buds. Meristematic somatic cells of male floral buds showed the best prometaphase which on (2*n* =30) chromosome complement was observed. Two chromosomes bearing satellites showed evident and large secondary constrictions. For the genetic diversity assessment, 61 genotypes were characterized using 6 SSR microsatellites. A total of 26 alleles were amplified. The effective number of alleles ranged between 1.92 and 4.34. Allele frequencies ranged from 0.008 to 0.68. Eight alleles (30.8%) observed as rare alleles. The observed heterozygosity (Ho) ranged from 0.35 to 0.67, while the expected heterozygosity (He) ranged from 0.48 to 0.77. Five of six loci showed an overall heterozygote deficiency; however, none of the assayed loci displayed a significant heterozygote deficiency. Two loci showed significant departure from Hardy–Weinberg equilibrium (HWE), while no significant deviations ($P<0.01$) from the Hardy–Weinberg proportions were found for four loci. Four of six markers were classified as very informative markers. Molecular analysis of variance (AMOVA) showed moderate differentiation in allele frequencies between four groups of populations and that 87.65% of variation is originated from variability between genotypes. Cluster analysis based on the euclidean distance using 'Unweighted Pair Group Method using Arithmetic Averages' (UPGMA) leaded to observe divergences between genotypes within site and between sites. The current study indicated an evident diversity probably due to a genetic flow between populations.

Keywords: *Pistacia atlantica* Desf. subsp. *atlantica*; Northwest Algeria ; morphological variability; climatic characterization; identification key; essential oils; cell wall polysaccharides; genome size; flow cytometry; chromosome number; genetic polymorphism; microsatellites markers.

RÉSUMÉ **APPROCHE MULTIDISCIPLINAIRE POUR LA CARACTERISATION DE LA DIVERSITE CHEZ *Pistacia atlantica* Desf. subsp *atlantica* EN ALGERIE NORD-OCCIDENTALE**

La présente étude porte sur la caractérisation de la diversité chez *Pistacia atlantica* Desf. subsp *atlantica* en Algérie Nord-occidentale.

Seize populations naturelles de *Pistacia atlantica* Desf. subsp. *atlantica* ont été prospectées sur un gradient longitudinal. Les données pluviothermiques des sites prospectées ont été utilisées pour la caractérisation climatique. L'étude a examiné morphologiquement 10 arbres matures par sites (mâles et femelles) et morphométriquement 3520 feuilles composées matures. Une corrélation significative entre les paramètres climatiques et la morphologie des feuilles a été montrée. Nous notons pour la première fois sur les échantillons observées que la longueur et la largeur de la feuille atteint jusqu'à 24,5 cm / 21,9 cm, le nombre des folioles jusqu'à 18 folioles / feuille et la longueur du pétiolule de la foliole terminale atteint jusqu'à 3,4 cm. Ces mesures nous ont permis de proposer et mettre à jour la clé d'identification de *Pistacia atlantica* subsp. *atlantica*. La variabilité intraspécifique chimique a été évaluée par la caractérisation des huiles essentielles des feuilles et des polysaccharides pariétaux. Les huiles essentielles ont été extraites par hydrodistillation puis analysées par chromatographie en phase gazeuse (GC) et chromatographie couplée à la spectrométrie de masse (GC/MS). Le rendement a varié de 0,1% à 0,42%. Terpinene-4-ol, α-pinène, germacrène D et *E*-caryophyllène ont été observés comme composants majoritaires. Les deux derniers composés n'ont jamais été mentionnés comme constituants majoritaires pour l'huile essentielle de cette espèce. L'analyse multivariée a montré 3 types d'huile essentielle compte tenu des constituants prédominants: 1) Terpinène-4-ol ; 2) α-pinène/camphène; 3) α-pinène/germacrène D. La cellulose et les hémicelluloses pariétales ont été isolées et dosées avec des rendements variant de 21,25% à 51% et de 7% à 27,6% respectivement à partir d'un rendement variant de 36,2% à 69,5% de résidu pariétal. L'estimation de la taille du génome a été effectuée par cytométrie en flux. L'intensité de fluorescence du iodure de propidium (IP) montrent que tous les échantillons analysés sont diploïdes (2n = 2x). La cytométrie en flux a permis d'estimer un très petit génome (2C = 1,21 ± 0,02 pg). Aucune différence statistique n'a été enregistrée entre les tailles génomiques des 97 échantillons analysés. Des préparations de 'Squash' pour le dénombrement chromosomique ont été réalisées sur des bourgeons floraux fixés. Les celllules somatiques méristématiques de bourgeons de fleurs mâles ont montré les meilleures prométaphases sur lesquelles le nombre (2n = 30) a été observé. Deux chromosomes présentant des satellites ont montré de constrictions secondaires grandes et évidentes. Pour l'évaluation de la diversité génétique, 61 génotypes ont été caractérisés par 6 microsatellites SSR. Un total de 26 allèles a été amplifié. Le nombre d'allèles effectifs a varié de 1,92 à 4,34. Les fréquences alléliques ont varié de 0,008 à 0,68. Huit allèles (30,8%) ont été observés comme des allèles rares. L'hétérozygotie observée (Ho) a varié de 0,35 à 0,67, tandis que l'hétérozygotie attendue (Ha) a varié de 0,48 à 0,77. Cinq des six loci ont montré un déficit global d'hétérozygotie; cependant, aucun des loci analysés n'a affiché un déficit important. Deux loci ont montré un écart important par rapport à l'équilibre Hardy-Weinberg (HWE), tandis qu'aucun écart significatif ($p < 0,01$) par rapport aux proportions de Hardy-Weinberg n'a été montré pour quatre loci. Quatre des six marqueurs ont été classés comme des marqueurs très informatifs. L'analyse moléculaire de la variance (AMOVA) a montré une différenciation modérée dans les fréquences alléliques entre les quatre groupes géographiques et que 87,65% de la variation est originaire de la variabilité entre les génotypes. L'analyse basée sur la distance euclidienne en utilisant la méthode (UPGMA) a permis d'observer des divergences entre les génotypes à l'intérieur du site et entre les sites. La présente étude indique une diversité évidente probablement dû à un flux génétique entre les populations.

Mots clés: *Pistacia atlantica* Desf. subsp. *atlantica*; Algérie Nord-Occidentale ; caractérisation climatique; variabilité morphologique; clé d'identification; huile essentielle; polysaccharides pariétaux; taille du génome; cytométrie en flux ; nombre de chromosome; polymorphisme génétique; marqueurs microsatellites.

TABLE OF CONTENTS

LIST OF ACRONYMS

A.H: *Anno Hegirae* (Hijri year)
AC: Aldehyde compounds
AFLP Amplification Fragment Length Polymorphisms
AFNOR : Association Française de Normalisation
AMOVA Molecular analysis of variance
ANOVA : Analysis Of Variance
APG: Angiosperm Phylogeny Group
Comb. Nov. : - combinatio nova (new combination)
CTAB : CetylTrimethyl Ammonium Bromide
DGF : Direction Générale des Forêts
DNA: DeoxyriboNucleic Acid
Eur. Ph : European Pharmacopoeia
FCM : flow cytometry
FID : flame ionization detector
GC*:* Gas chromatography
GC–MS: Gas chromatography–mass spectrometry
HAC : Hierarchical Ascendant Classification
HM: Hydrocarbon monoterpenes
HP GC : Hewlett Packard Gas chromatography
HS: Hydrocarbon sesquiterpenes
HWE: Hardy–Weinberg equilibrium
IPCC: Intergovernmental Panel on Climate Change
IPGRI: The International Plant Genetic Resources Institute
IPNI : International Plant Name Index
ISO : International Standard Organization
ISSR: Inter-Simple Sequence Repeat
OM: Oxygen containing monoterpenes
ONM : Office National de Météorologie
OS: Oxygen containing sesquiterpenes
PCA: Principal Components Analysis
PCR : Polymerase Chain Reaction
RAPD : Randomly Amplified Polymorphic DNA
RFLP : Restriction Fragment Length Polymorphisms
SAMPL : Selective Amplification of Microsatellite Polymorphic Loci
SNP : Single Nucleotide Polymorphisms
SRAP : Sequence-Related Amplified Polymorphism
SSLP : Simple Sequence Length Polymorphism
SSR Simple Sequence Repeat
Stat Soft.Inc: Statistica software Incorporation.
STR : Short Tandem Repeats
UNEP-GEF: United Nations Environment Programme- Global Environment Facility.
UPGMA Unweighted Pair Group Method with Arithmetic Mean
USDA/ARS/GRIN: Unated States Department of Agriculture /Agriculture Research Service/Germplasm Resources Information Network.

LIST OF ABBREVIATIONS

cpDNA: Chloroplastidic DeoxyriboNucleic Acid
dc: duct
d.f: degree of freedom
EO essential oil
f. forma (form)
Fl. Atlant: Flora Atlantica
FU/kg: Fodder units Kilograms
kV .. s : applied voltage for separation
Mbp: mega base pairs = 1,000,000 bp
r.p.m.: revolution per minute, rotation per minute
s.d: standard deviation
SS: Sum of squares 0.33
subsp:subspecies
ssp. species
UV: ultraviolet

LIST OF AUTHOR NAME ABREVIATION IN BOTANY

Boiss. Boissier
Desf. : Desfontaines
Engl. : Engler
Fisch. Fischer
Franch.: Franchet
L. Linnaeus
Poiss. Poisson
Sarg. Sargant
Zoh.: Zohary

LIST OF FIGURES

Chapter 1

Chapter 5

Chapter 6

Chapter 7

LIST OF TABLES

INTRODUCTION

INTRODUCTION

The Mediterranean region is situated between Laurasia and the Gondwana vestiges where vegetation is originated from both areas (Quezel, 1960; 1985; Suc *et al*., 1995; Pons and Quezel, 1998; Fauquette *et al*., 1999). The flora in this region was predominantly Mediterranean with a number of tropical and temperate elements (Le Houerou, 1997). The Mediterranean region has known between -3.5 Ma and -2.6 Ma a subsequent dry phases which modified the Mediterranean Landscape (Suc, 1984; Suc *et al*., 1995). The climatic changes following the last glacial retreat have greatly influenced the vegetation structure in the region (Bazile-Robert *et al*., 1980). In North Africa, those changes favored the installation of thermo and meso-mediterranean vegetation to the detriment of some taxa which are mostly in extinction or which have found a refugium in particular zones (Van Campo, 1975; Van Zinderen Bakker and Maley, 1979; Maley, 1980; El Hamouti *et al*., 1991; Stambouli-Essassi, 2003; Benslama *et al*., 2010). Climate change that occurred from Holocene to Upper Pleistocene epochs had modified the vegetation structure; however, many paleoecological studies showed that Man had shaped the nature and especially the vegetation (Frenzel, 1979; Leveau *et al*., 1998) jointly with the contemporary climatic changes (Le Houerou, 1991). Actually, the deepest studies on the global warming in the Mediterranean region remain insufficient to draw conclusions (Quereda-Sala *et al*., 2000; IPCC, 2001; Alpert *et al*., 2002; UNEP-GEF, 2002; Ulbrich *et al*., 2005; Benslama *et al*., 2010).The thermal value reported on the global warming in the Mediterranean region remains modest; however, the impact on the vegetation would not be negligible (Agoumi, 2003; Medail and Quezel, 2003).

Even if the plant responses to environmental changes cannot be observed on all the plant communities, they can be viewed individually on the species (Huntley, 1991; Birks and Line, 1993). Many studies are focused on the impact of climatic changes on the genetic variability and the long-term survival of living beings (Asch *et al*., 2007; Willis *et al*., 2008; 2010; Hoffmann and Sgrò, 2011). This issue is the most important since the current fragmentation of natural habitats strongly restricts the opportunities of the dispersal of species, as well as, the gene flow among populations. When species exhibits a large range, the fragmentation of the habitats and the adaptive potentialities to the ecological variability may lead to intra-specific subdivisions, breeding subspecies, varieties, and ecotypes. This is the case of the Atlas Pistachio, *Pistacia atlantica* Desf. [Fl. Atlant. 2:364. 1799] (Desfontaines, 1799; 1800). It is an Irano-Touranian species with a large geographic range (Zohary, 1952). Irano-Touranian plants would have existed in the Mediterranean region during the dry and the cold climatic phases of the Miocene (Benslama

et al., 2010; Biltekin *et al*., 2015). The Plio-Pleistoncene glaciations were propitious for their establishment in the region. Even if its range is more or less discontinuous, *Pistacia atlantica* is one of the most widely distributed wild species of the genus. It occurs from the Canary Islands to Pamir Mountains (Zohary, 1952). Over this large area and under different ecological conditions, *P. atlantica* populations adapt differently; the morphological characters of the species are very variable, leading to taxonomic confusion mainly in the infraspecific level. Until then, 3 subspecies are admitted (*P. atlantica* subsp. *atlantica*, *P. atlantica* subsp. *cabulica*, *P. atlantica* subsp. *mutica*) (Yaltirik, 1967a; 1967b; Al yafi, 1979), while Rechinger (1969) and Al yafi (1978) recognized *P. atlantica* subsp. *kurdica* (already described by Zohary (1952)) as a subspecies. This taxon is considered by Yaltirik (1967a; 1967b) and Al yafi (1979) as a distinct species.

Pistacia atlantica's subspecies are qualified as eco-geographical ecotypes (Rechinger, 1969; Browicz, 1988). Morphologically, they are related by an intermediaries and they transgress geographically, more or less, the areas assigned to them (Monjauze, 1968). In fact, the subspecies *atlantica* which is qualified as the North-African representative (Zohary, 1952) and considered to be native to Maghreb countries (Browicz, 1988) is nevertheless cited among the subspecies studied in the Asiatic area of the species (eg. Syria and Turkey) (Karimi *et al*., 2009).

Problematic and objectives

As for the species '*Pistacia atlantica'*, within the extensive region of its subspecies, the taxon is subject to a significant variability in environmental conditions. In order to respond effectively to the different living needs, the subspecies adopts several adaptive strategies which have interested plenty of studies mainly in Algeria. The leaf morphological variability was investigated in the Algerian central steppe (Belhadj *et al*., 2008), in the region of Tiaret (Dahmani, 2011; Mehdeb, 2011) and in the western Algerian Sahara (region of Bechar, Ait Said *et al*., 2011a). Important pollen shape and size diversity has been revealed by Belhadj *et al.* (2007a). Phytodermological studies examined stomatal complex and trichomes in different regions in Algeria showing a high variability between the studied populations (Kadi Bennane *et al*., 2005; Belhadj *el al*., 2007b, 2008; Tirse *et al*., 2014). However, the origin of this variability was not deeply studied.

The morphological variability of *P. atlantica* in Algeria is a long-standing issue. Zohary (1952) reports the presence (in the Northwest and the Northeast of Algeria) of a variety where the leaves and the fruits are larger and look like those of the var. *latifolia* Zoh. Monjauze (1982) reports that the specimen of *Desfontaines herbarium* (DF-58/17; DF-58/20; see Bruns-Balogh, 1991) showed a microphyllious variety which is different from some specimens observed in the country. Monjauze (1982) reported also the possibility to have a different variety in the Algerian steppe

with a round and black fruits. He alluded to a certain resemblance to *P. atlantica* subsp. *kurdika* Zoh. More recently, Belhadj *et al.* (2008) queried the existence of any other varieties or subspecies in Algeria without, nevertheless, providing any answers.

The objective of the current thesis is to investigate the origins of the diversity showed by *Pistacia atlantica* in Algeria. The response to this issue requires a multidisciplinary study. Natural populations of *P. atlantica* subsp. *atlantica* in a large area in Northwest Algeria, where some populations which had aroused the curiosity of some famous botanists (Zohary 1952; Monjauze 1982), are the focus of this study.

Thesis design

The structure of the current thesis is as follows:

Chapter 1. State of the art review:
Pistacia L., *Pistacia atlantica* complex: taxonomic retrospection and monography

This section outlines a pertinent data on the taxonomic and monographic review of the genus *Pistacia* and especially the species *P. atlantica*. This section brings to date our knowledge through previous researches relevant to the topic of the evolution of the genus taxonomic status.
The botanical description and the ecological characteristics of *Pistacia atlantica* summarized in this chapter were useful firstly to guide the research aims and secondly to discuss the findings of this thesis. Convinced of their originality, the researcher added, to this chapter, personal photos from field collection hoping to enrich the species monography.

Chapter 2. Climatic characterization of *Pistacia atlantica* subsp. *atlantica* natural area in Northwest Algeria

The diversity study in spacio-temporal context is so important, mainly in the intraspecific level. The impact of climate on the observed variability could not be neglected. Thus, in the current study, the climatic factors in Northwest Algeria were targeted. The climatic characterization of the studied sites will be detailed in this chapter. The climatic conditions inform on the ecological characteristics of species. Thus, at the end of this chapter, the results of climatic characterization of the sites were discussed to better understand the factors influencing the dynamics of Pistachio populations.

Chapter 3. Morphological characterization and morphological variability analysis of *Pistacia atlantica* subsp. *atlantica* natural area in Northwest Algeria

The assessment of phenotypic variation focuses on morphological traits that define the shape and appearance of a set of individuals. In this chapter, the variability was assessed at the phenotypic level and the morphological characterization was checked within and between populations. Furthermore, we had to ascertain if the morphological variability is significant and what this variability might provide to the database of the subspecies and to its identification key.

In the light of the results achieved in chapter 2, climatic and morphologic data should be used to investigate if the climatic variation affects the leaf morphology of the species; but also what are the most important climatic parameters that influence Atlas pistachio variability. Climatic and morphological correlations will be assessed and discussed in this chapter.

Chapter 4 & Chapter 5

The multidisciplinary approach could be properly conducted if we examine also the biochemical profile of Pistachio populations. The produced metabolites of plants have very often served the taxonomic studies. Hence, the chemical variability of the essential oils and the cell wall polysaccharides will be investigated. The two aspects will be given in Chapter 4 and Chapter 5, respectively.

Chapter 4. Intraspecific chemical variability of *Pistacia atlantica* Desf. subsp. *atlantica* essential oil from Northwest Algeria

This chapter exposes the quali-quantitative and characterization of *Pistacia atlantica* subsp. *atlantica* essential oil, the inter-population chemical variability of *Pistacia atlantica* subsp. *atlantica* essential oil and the analysis of the environmental factors effect on the chemical variability.

Chapter 5. Quantitative characterization of cell wall polysaccharides from *Pistacia atlantica* leaves through cellulose and hemicelluloses dosage

This chapter reports preliminary quantitative and qualitative characterization of cell wall polysaccharides extracted from mature leaves. This study is, to our knowledge, the first to consider cell wall polysaccharides' valorization in *Pistacia atlantica*.

Chapter 6 & Chapter 7

We have to verify if the phenotypic variability depends on cytogenetic or genetic variability. For some years, the study of both the two aspects is possible in the intra-specific level using the molecular tools. The molecular characterization will be presented in chapter 6 and Chapter 7, respectively.

Chapter 6. Genome size determination and Chromosome assessment in *Pistacia atlantica* Desf. subsp. *atlantica* from Northwest Algeria

The chapter 6 exposes the cytogenetic characterization focusing on the determination of the genome size and the chromosome number of *Pistacia atlantica* subsp. *atlantica*. In this field, data is still missing for the genus and especially for the species. The few previous studies are, however, still confusing. The causes of these confusions will also be discussed in this chapter.

Chapter 7. Molecular characterization and evaluation of intraspecific genetic diversity of *Pistacia atlantica* Desf. subsp. *atlantica* in Northwest Algeria, using SSRs markers

The chapter 7 presents a contribution of genetic fingerprinting of *Pistacia atlantica* subsp. *atlantica* using molecular markers. The genetic characterization of the natural populations in Northwest Algeria taken as sample for the current study will contribute to assess the genetic diversity of this subspecies and to evaluate the interpopulational genetic relationships.

General conclusion and prospects

The purpose of this chapter is to discuss the overall results obtained in the different steps of this study. The findings of the current study are the proud achievement of more than five years of field investigation. The discussion helps us to understand the origin of diversity and to answer many previous questions about the morphological variability in Algeria. Nevertheless, it opens up new prospects for deep and detailed characterization in order to understand the structure of *Pistacia atlantica* subsp. *atlantica* natural populations in Algeria and its evolutionary history.

CHAPTER 1: STATE OF THE ART REVIEW: *Pistacia* L., *Pistacia atlantica*: TAXONOMIC RETROSPECTION AND MONOGRAPHY

1 STATE OF THE ART REVIEW: *Pistacia* L., *Pistacia atlantica:* TAXONOMIC RETROSPECTION AND MONOGRAPHY

1.0 Background

The origin of the genus *Pistacia* is still questionable. Based on fossil records (Weyland, 1941), Zohary (1952) supposed that the origin center of *Pistacia* species is placed in central and Southwestern Asia and that the genus as a whole probably developed more than 80 million years ago. Numerous archaeological excavations (Ramirez and Cevallos-Ferriz, 2002) report the finding of pistachio nuts in Neolithic settlements, West of the Zagros Mountains (mountain range situated in Western Iran) from the eight until the sixth millennium B.C. (*in* Hormaza, 1995). Nevertheless, other researchers did not agree with this hypothesis, since Anacardiaceae pollen and wood first appear in the Paleocene epoch, 65 to 55 million years ago and is found throughout the world (Hsu, 1983; Muller, 1984). The origin for the order in which the Anacardiaceae occurs, Sapindales, dates back approximately 84 to 65 million years before present (Magallon and Sanderson, 2001; Wikstrom *et al*., 2001).

In fact, Al-Saghir (2006; 2009) hypothesizes that *Pistacia* originated in the Paleocene epoch and postulates that ancestral species of *Pistacia* came from North America since, Anacardiaceae is pantropical in its distribution and North and South America represent major diversification centers of the family. Given the geographical distribution of *Pistacia*, Al-Saghir's hypothesis (Al-Saghir, 2006; 2009) is supported by *Pistacia* fossil records from the Paleocene of Wyoming and Colorado (Edwards and Wonnacott, 1935). Migration may have taken place from Western Laurasia (North America) to Eastern Laurasia (Europe and Asia) ending up in Central Asia via Europe where the genus radiated within Asia (West Asia and Mediterranean Basin) as hypothesized by Weeks *et al.* (2005) for the Burseraceae (a family closely related to Anacardiaceae). This migration may have been facilitated by the boreotropical land bridge (Tiffney, 1985; Tiffney and Manchester, 2001), which spanned the North Atlantic during the early to middle Eocene. Global temperatures during the Eocene were highest during this time period and tropical vegetation is known to have occurred in this land corridor (Wolfe, 1978; Zachos *et al.*, 2001). Cooler temperatures during the Middle Eocene extirpated frost tolerant taxa in this region and the physical land connections disappeared sometime afterward (Weeks *et al*., 2005). Al-Saghir's hypothesis (Al-Saghir, 2006; 2009) may also be supported by the cladistic analysis of NIA-i3 gene region which showed astonishing close relationships between the *P. atlantica* - *P. khinjuk* - *P. vera* clade and the *P. mexicana* - *P. texana* clade (Yi *et al*., 2008). The last two species represent the genus in America (Zohary, 1952).

Nevertheless, it is believed that the Eastern part of Zagros Mountains is one of the main centers of diversity for *Pistacia.* However, the pistachio nuts found in Neolithic settlements in the region and reported as true pistachio nuts (from *P. vera*) could easily be nuts from other *Pistacia* species such as *P. palaestina* or *P. atlantica* which are indigenous to those areas and whose seeds are eaten by local people (Hormaza, 1995). The genus extended its distribution range by passive dispersal mediated by wind, water, birds or even by people (Al-Saghir, 2006). This is supported by evolution toward a smaller seed with a hard endocarp paralleling a change in reproductive strategy from distribution by ground squirrels (burying the seed, as with walnuts and oaks) to bird- or wind-mediated distribution, which would require a seed capable of passing through a bird's digestive system or being blown by the wind (Jordano, 1989). The name pistachio probably derives from the word Pista-pistak in the ancient Persian language Avestan (Joret, 1976).

1.1 *Pistacia atlatica* 'El Buttum' in the history of mankind

It is beautiful in its greatness, it is generous by its products, it touched the human life since the old history. Atlas pistachio, called in arabic 'El Buttum' is cited many centuries before Christ, the old writing stands witness (Jung, 1954). Additional references to Atlas pistachio are found in the bible (eg. Genesis 35:4). Jung (1954) reports that these trees have been from ancient time symbols of the Semetic love and mother goddess, that oaks and terebinths[1] (turpentine tree) are, in the Old Testament, oracle trees.

According to Ibn Kathir [≈ 700 - 774 A.H], in the tale of the prophet Yusuf [Joseph (pbuh)], he reported that the prophet Yusuf was rescued from the well by traders of pistachio, pine and Atlas pistachio fruits. It is also reported that in the ancient Egypt, a guarded missions were sent to look for a honey in the pistachio stands in the Sahara (Adra, 2011). In addition, according to historians, Atlas pistachio was planted in the gardens and the temples of the Pharaohs to benefit from the trunk exudates such as the resin used as chewing gum and mainly in their receipts of aromatherapy and mummification procedure (Al Dassouki, 2011). Furthermore, it is mentioned in the historical Islamic texts that somewhere in the Syrian Desert, an Atlas pistachio tree has shaded the prophet Muhammad $^{(SAW)}$(as young boy) on one of the trade journey from Mecca to Syria (Adra, 2011).

Because of its large size and great age, 'El Buttum' trees were well-known landmarks and were used as memorials for the dead (Fig. 1.1 and Fig. 1.2), a practice followed until recently in some

[1] The noun 'terebinth' cited on the references translated from the ancient languages refers to *Pistacia atlantica*; many authors relied on the botanical description of the tree to substantiate their arguments.

Arab villages (Middle East and Maghreb). In Cyprus (Italy), an old city with a temple was called Tremithounta (Ôñåìéëïýíôá in Greek) from the Greek name of *Pistacia atlantica* (Elizabeth, 2008). In Algeria, the spiritual statute of Atlas pistachio has often contributed to the preservation of the species.

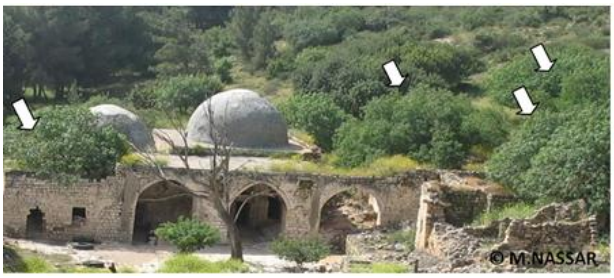


Fig. 1.1 One of the memorials of the prophet Yusha [Joshua (pbuh)] in Atlas pistachio stand (Safad- Palestine, Mars, 2005) (Webmaster1)

Fig. 1.2 A saint's tomb and a cemetery in Atlas pistachio stand (Betaim- Tlemcen Algeria, Mars 2013)

1.2 *Pistacia* L.: Evolution of taxonomic status

Pistacia atlantica has known a long taxonomic history and several revisions in all its higher hierarchical levels. Before the identification of *Pistacia atlantica*, the other members of the genus were grouped separately by Tournefort (1700) into two genera (*Lentiscus* and *Terebinthus*). In his '*Species Plantarum'*, Linnaeus (1753) was the first to establish, officially, the genus ***Pistacia***.

The class level

In '*Systema Naturæ'*, *Linnaeus* (1735-1758) places the genus into the class *Dioecia*, *Pistacia* has been merged, afterwards, into many classes: *Terebinthineæ* (Spach, 1834; Endlicher, 1836-1840), *Magnoliopsida* (Subclass: Rosidae) (Cronquist, 1981), (Subclass: *Malvidaæ*) (Throne and Reveal, 2007). The Angiosperm Working Group (APG, 1998, 2003, 2009) places the genus in Eudicots; core Eudicot; Rosid; **Eurosid II**.

The order level

Linnaeus (1735-1758) places the genus into the order *Pentandria.,* then the genus appears with the oak trees and the walnut trees into the order '*Amentaceæ*[2]'. This classification was done on the base of the resemblance of some characters of the flowers (reduced, small and unisexual flowers).

[2] The endings 'ceæ' was given in this classification for the orders.

Subsequently, several orders have been proposed (*Terebinthinæ*, *Terebinthaceæ*[2], Terebinthales, Sapindales, Rutales and Burserales) (Table 1.1).

Table 1.1 Proposed ordinal affinities of *Pistacia* based on morphological or molecular data

Order	References	
	Morphological data	**Molecular data**
Amentaceæ[2]	*Linnaeus* (1735-1758)	
Rutales	Gundersen, 1950; Thorne 1992	
Burserales	Takhtajan, 1997	
Sapindales	Engler, 1892; Rendle, 1925; Hutchinson, 1926; 1973; Takhtajan, 1954; Dahlgren, 1980; Cronquist, 1968, 1981, 1988; Bhattacharyya and Johri, 1998.	Chase *et al*., 1993; Gadek *et al*, 1996; APG 1998, 2003; 2009; Bremer *et al*., 1999, Savolainen *et al*., 2000; 2000
Terebinthaceæ[2]	A.L. De Jussieu (1789)	
Terebinthinæ	Eichler (1875-1878) ; Hallier, 1908 (*Terebinthinæ*); Wettstein, 1935 & 1944 (Terebinthales)	

Modified from Pell (2004)

The **Sapindales** are mostly woody plants with a synapomorphic prominent nectariferous disc and a syncarpous gynoecium usually with one or two ovules per locule (Gadek *et al*., 1996). Besides these features, the recent molecular studies retain the classification of the genus into the Sapindales (Fig. 1.3) (Gadek *et al*., 1996; Chase *et al*., 1993; APG, 1998, 2003; 2009; Bremer *et al*., 1999; Savolainen *et al*., 2000a, 2000b, Stevens, 2008).

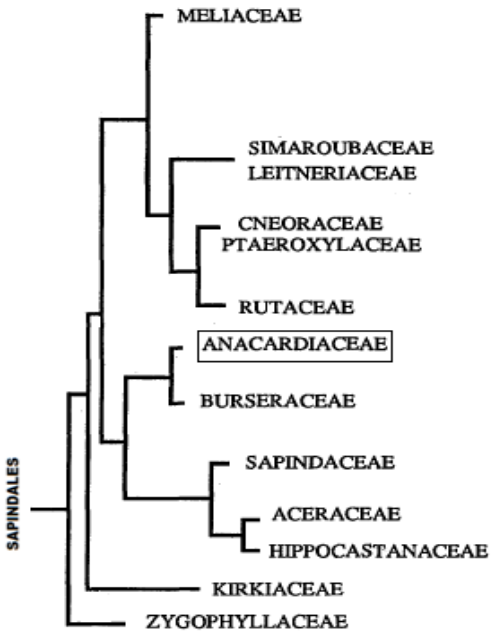


Fig.1.3 Position of the Anacardiaceae family (framed) in the phylogenetic tree of the Sapindales order (modified from Gadek *et al*., 1996)

The Family level

Based on morphological details, the genus *Pistacia* was firstly the unique representative of its own family, *Pistaciaceæ* by Adanson (1763). Many studies showed the particularity of certain characteristics of the genus. *Pistacia* species are dioecious, have reduced perianth, unusual pollen

morphology (with up to eight apertures, and plumose styles with associated increased stigmatic surface area) (Erdtman, 1971; Mabberley, 1997; Pell, 2004; Belhadj *et al*., 2007a). All These morphological features are adaptations for wind pollination. In 1825, De Candolle, working on the order of *Terebinthaceæ* of A. L. Jussieu (1789), establishes the Family '*Terebinthaceæ'*(cited 'Térébinthacées' in his 'Mémoire sur la famille des légumineuses'). In 1830, Lindley, in his 'An Introduction to the natural system of botany', states that he agree with the botanists who abandon the name '*Terebinthaceæ'*. He validates, so, the family of the *Anacardiaceæ*. This family was firstly proposed by Brown (1818) who constitutes it by the species related to the *Anacardium*. Lindley (1830) gives the diagnosis, the anomalies, the essential character, the affinities, the geography and the properties of the validated family and cites the genus *Pistacia* as member of this family with the Cashew and the Mango. Working on the class of *Terebinthinaæ* Barling, Spach (1834) includes the genus *Pistacia* in the family of *Cassuvieæ*. This opinion was not adopted for a long time. Lindley's classification dominates. The flowers of the ***Anacardiaceæ*** are generally not highly prominent but are discerning by an intrastaminal nectariferous disc. Based on the synapomorphies of a single apotropous ovule (an ovule with a raphe that is ventral when ascending and dorsal when descending, Fig. 1.4). The morphological data exposed by Wannan and Quinn (1991) and many molecular data (Pell, 2004; Yi *et al*., 2004; 2007) support this classification.

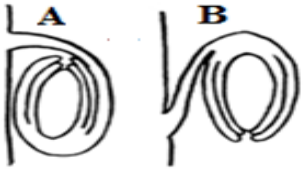


Fig. 1.4 Apotropous ovule (as found in Anacardiaceæ):
A): descending ovule with a dorsal raphe;
B): ascending ovule with a ventral raphe (Redrawn and modified by Pell (2004) from Geesink *et al*., 1981)

<u>The infrafamilial level (Subfamily and Tribe)</u>

Eichler (1875-1878) treated *Pistacia* as a distinct group. Takhtajan (1997) divided the Anacardiaceae into four subfamilies (*Anacardioideæ* Link, *Spondioideæ* Link, *Julianioideæ* and *Pistacioideæ* Burnett.) and puts the genus *Pistacia* in the last one. On the basis of wood anatomy, fruit and flower morphology, and flavonoid chemistry, Wannan and Quin (1991) divided the *Anacardiaceae* into two groups, A and B. These groups overlap the subfamilies *Spondioideae* and *Anacardioideae* within molecular studies of Terrazas (1994) and Pell (2004). *Pistacia* was placed in the subfamily ***Anacardioideae*** in both studies. The genus *Pistacia* was classified in the tribe *Anacarediæ* by certain authors as Spach (1834) and De Candolle (1928), then treated as a distinct tribe or a subfamily (Pistacioideae) (Marchand, 1869; Eichler, 1875 1878; Takhtajan, 1987, 1997; Mitchell *et al*., 2006). Engler (1876) placed *Pistacia* in the tribe *Rhoideæ* (= *Rhoeæ*). This

treatment was followed by Engler (1883, 1892). The *Rhoeae* was cited by Takhtajan (1997) included within the subfamily *Spondioideæ*. The most widely accepted classification divides the *Anacardiaceae* into five tribes: *Anacardieae, Semecarpeae, Spondiadeae, Dobineae* and *Rhoeae* (Mitchell and Mori, 1987; Wannan and Quinn, 1991). *Pistacia* was assigned to the last one (Mitchell and Mori, 1987); in fact, it resembles the *Rhoeae* members by having three syncarpous carpels, unilocular fruits, and a thin exocarp. If the last subfamily classification (Terrazas, 1994; Pell, 2004) and the last tribe classification (Mitchell and Mori, 1987; Wannan and Quinn, 1991) are the most accepted, the *Rhoeae* could not be included within the *Spondioideae* (*sensus* Takhtajan, 1997) but in the *Anacardioideae* (*sensu* Terrazas, 1994 ; Pell, 2004). Terrazas (1994) and Pell (2004) placed *Pistacia* in the subfamily Anacardioideae.

The genus level

As it was mentioned above, *Linnaeus* (1753) was the first to establish the genus, recognizing six species: *Pistacia lentiscus* L., *P. terebinthus* L., *P. vera* L., *P. narbonensis* L., *P. trifolia* L. *and P. simaruba* L. Many other species was added to the genus; Desfontaines (1799) described *P. atlantica* while Humboldt *et al*. (1824) described *P. mexicana* as a new species. Marchand (1869) included to the genus four species (*P. chinensis* Bunge. (described by Bunge (1835)), *P. mutica* Fisch. (described by Fischer and Meyer (1838)), *P. Khinjuk* Stocks (described by Stockmans (in Stockmans and Hooker, 1852)) and P. *palaestina* Boiss. (described by Boissier (1849)). Engler (1883) provides the first monograph of the genus and withdraws from *Linnaeus's* list: *P. trifolia* (considered as synonym of *P. vera*) and *P. narbonensis* (considered as an hybrid between *P. vera and P. terebinthus*). He suggests that *P. palaestina* is a subspecies of *P. terebinthus;* when *P. simaruba* is *Bursera simariba* (L.) Sarg., member of the closely related *Burseraceæ*.

Subsequently, many species are described and added to the genus *Pistacia*: *P. falcate* (Martelli, 1886) *P. weinmannifolia* (Franchet, 1886) and *P. texana* (Swingle, 1920). It is known that the first and the most complete classification of the genus is done by Zohary (1952). He considers 11 species for the genus and puts them, on the basis of the morphology of leaves (Fig. 1.5), leaflet, inflorescence, flowers, fruits, and the seedlings, into four sections: ***Lenticella* (Zoh.)** (Sub-persistent leaves, including *P. mexicana , P. texana* Swingle), ***Eu Lentiscus* (Zoh.)** (Persistent and paripinnate leaves, including *P. lentiscus, P. saportae* Burnat, *P. weinmannifolia* Poiss. ex Franch.), ***Eu Terebinthus* (Zoh.)** (Deciduous and pari/imparipinnate leaves, including *P. chinensis, P. khinjuk, P. palaestina, P. terebinthus, P. vera*) and ***Butmela* (Zoh.)** (deciduous and imparipinnate leaves, where *Pistacia atlantica* is the only member).

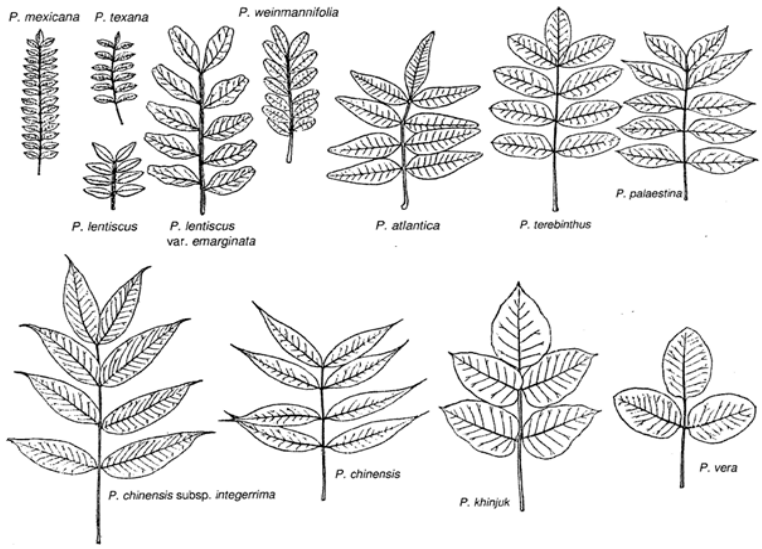


Fig.1.5 Leaf morphology of the various species in the genus *Pistacia* L. (adapted from Zohary M., 1952 by Zohary D. 1996)

Beside the leaf and seed morphological characters, on the basis of restriction fragment length polymorphism analysis of the *Pistacia* cpDNA, Parfitt and Badenes (1997) suggest to divide the genus into two sections (Fig. 1.6), ***Lentiscus*** and ***Terebinthus***. Section *Lentiscus* (including Zohary's sections *Letiscella* and *Eu Lentiscus*) consists of the evergreen species with paripinnate leaves and smaller seeds. Section *Terebinthus* (*Butmela* and *Eu Terebinthus* Zohary's sections) includes the deciduous species with imparipinnate leaves and large seeds.

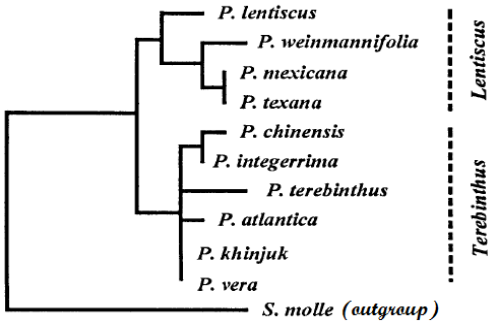


Fig. 1.6 Phylograms from mixed Dollo parsimony analyses of 10 *Pistacia* species and *Schinus molle* as outgroup (Parfitt and Badenes, 1997)

Many molecular studies support this division (Kafkas and Perl-Treves, 2001, 2002; Golan-Goldhirsh *et al*., 2004; Kafkas, 2006). Zohary (1972) revises his last classification and suggests *P. saportae* to be an interspecific hybrid. The genus *Pistacia* has known after Zohary's classification several new species. The taxonomic status of the genus members has been continually reviewed. Yaltirik (1967a, 1967b) adds a new species (*P. eurycarpa*) which was described by Zohary (1952) as *P. atlantica* var. *kurdika.* The data shown by the phenotypic cluster analysis made by Kafkas and Perl-Treves (2001) and AL-Saghir (2010a) confirms the taxonomic placement of *P. eurycarpa* as a separate species. Yaltirik (1967a, 1967b) considers *P. palaestina* as a variety of *P. terebinthus*. In fact, the same consideration was done by Engler (1883); but *P. palaestina* has been raised to the rank of a species by Zohary (1952). Yaltirik's opinion is shared by other authors suggesting close relationships between the two species; the two entities are morphologically,

ecologically and genetically similar (Zohary, 2000; Kafkas and Perl-Treves, 2002; AL-Saghir, 2010a). This opinion is supported mainly by a molecular data (Golan-Goldhirsh *et al*., 2004; AL-Saghir and Porter, 2006; Yi *et al*., 2008). According to Kokwaro and Gillett (1980), a new species from East Africa, *P. aethiopica* Kokwaro, is described by Kokwaro. However, its status has not been confirmed. This taxon was described by Zohary (1952) as a variety of *P. lentiscus.* AL-Saghir (2006) supports Zohary's classification considering *P. aethiopica* as *P. lentiscus* var. *emarginata.* Then, AL-Saghir and Porter (2012) suggest that this taxon should be treated as a subspecies of *P. lentiscus,* not as a variety (*Pistacia lentiscus* subsp. *emarginata* (Engl.) AL-Saghir, Comb. Nov.). Other species are cited in the different studies concerning the genus. Their taxonomic position within the genus remains to be discussed. *P. integerrima* is considered as a separate species (Parfitt and Badenes, 1997; Yi *et al*., 2008) but as a subspecies of *P. chinensis* (AL-Saghir and Porter, 2012). Finely, it is reported (AL-Saghir and Porter, 2012) that fifty-five binomials for *Pistacia* species were listed in the International Plant Name Index (IPNI, 2010). Nevertheless, most of them are not recognized. The most important studies which treats the systematic of the genus admit the following list: *P. chinensis* (including the subspecies: *chinensis, falcate, integerrima*), *P. eurycarpa, P. khinjuk, P. lentiscus* (including the subspecies *emarginata, lentiscus*), *P. mexicana, P. palaestina,P. terebinthus, P. texana, P. vera, P. weinmannifolia. P. atlantica* (including the subspecies: *atlantica*, *cabulica*, and *mutica*).

1.3 *Pistacia atlantica:* Evolution of taxonomic status

As cited previously, Man has known *Pistacia atlantica* for very long. However, the botanists before 1779 have always considered it as a variety of *Pistacia terebinthus.* The distinction between the two taxa was done by René Louiche Desfontaines when he saw the tree in Gafsa (Tunisia), during his botanical exploration he led in Tunisia and Algeria from 1783 to 1786. He published so '*Flora Atlantica*' (Desfontaine, 1798; 1800) (Fig. 1.7) where he described the species and gave it the scientific name *Pistacia atlantica* according to the Atlas Mountains (Fig. 1.8) of the Maghreb (Desfontaines, 1799, 1800). Later, De Candolle (1825) and Engler (1883) recognized the species in the revision of the genus. Nevertheless, even after Desfontaines's description, and Engler's monograph a degree of confusion still reigns and many explorers, mainly in the Maghreb, reported El buttum as 'Terebinthe' and cited it as a subspecies or variety of *Pistacia terebinthus.* Battandier and Trabut (1888) described in the genus *Pistacia* the two species separately however; *P. atlantica* is cited again as a subspecies of *P.terebinthus* by Battandier and Trabut (1902). Then in 1910, Battandier noted that *P. atlantica* should be treated as a variety of *P terebinthus.*

364 DIOECIA PENTANDRIA.

Pistacia. *Camer. Epit.* 170. *Ic.—Matth. Com.* — *Adv.* 412. *Ic.* — *Dod. Pempt.* 817. *Ic.* — *Hist.* 361. *Ic.—J. B. Hist.* 1. *p.* 275. *Ic.* —
Le Pistachier. *Regnault. Bot. Ic.*

COLITUR in regno Tunetano. P. trifoliata videtur. ♄

PISTACIA TEREB

PISTACIA foliis impari-pinnatis; foliolis ovat
Terebinthus vulgaris. *C. B. Pin.* 400. — *T. Ic.* — *Matth. Com.* 108. *Ic.—Dod. Pempt.* *Tabern. Ic.* 1025. — *Camer. Epit.* 51. *Ic.* *t.* 87. — *Schaw. Specim. n.* 573.
Le Térébinthe. *Regnault. Bot. Ic.*

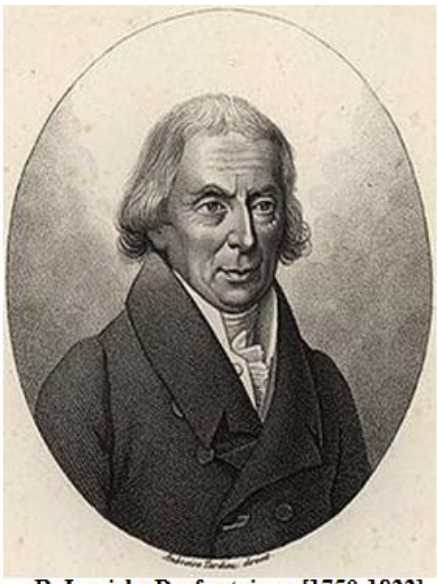
HABITAT Algeriâ in montibus. ♄

FLORA ATLANTICA,
SIVE
HISTORIA PLANTARUM,
QUÆ IN ATLANTE, AGRO TUNETANO
ET ALGERIENSI CRESCUNT.
AUCTORE RENATO DESFONTAINES, Instituti nationalis Scientiarum Galliæ socio, necnon in Museo Historiæ naturalis Parisiensi Botanices professore.
TOMUS SECUNDUS.
PARISIIS,
Apud editorem L. G. DESGRANGES, in vico Haute-Feuille, N° 14.
Typis C. PANCKOUCKE.
ANNO SEXTO REIPUBLICÆ GALLICÆ.

PISTACIA ATLANTICA.

PISTACIA foliis deciduis, impari-pinnatis; foliolis lanceolatis, subundulatis; petiolis alatis.

ARBOR 15—20 metr.; trunci diametro 9—12 decimetr. Rami sylvosâ multitudine in comam densam, latissimam, subrotundam emergunt. Cortex in ramulis griseus. Folia decidua, impari-pinnata; foliolis septem ad novem, angusto-lanceolatis, glaberrimis, integerrimis, margine undulatis, subrepandis, obtusiusculis, 2—3 centimetr. longis, 6—8 millimetr. latis. Petiolus superne compressus, alâ brevissimâ hinc et inde cinctus. Flores dioici. Masculi terminales, in racemos thyrsoideos dispositi. Squama parva, ovata, decidua, superne excavata, sericea ad basim singuli ramuli. Calyx tri aut quinquepartitus; laciniis subulatis, pubescentibus. Corolla nulla. Stamina 5, rarius 7. Filamenta brevissima. Antheræ oblongæ, bilobæ, obtusæ, intense rubræ, polline flavo fœtæ. Fœminei laxe racemosi; racemis paniculatis. Squamulæ angustiores quam in maribus. Calyx minimus, tri aut quinquepartitus; laciniis acutis. Corolla nulla. Styli 3, purpurei, sæpe inæquales. Stigmata totidem, crassiuscula. Germen minimum, ovoideum. Drupa subcarnosa, subrotunda, magnitudine fere Pisi, ante maturitatem flavo-purpurea, matura cœrulescens, nucleo subrotundo fœta. Folia in junioribus individuis elliptica observavi.



R. Louiche Desfontaines [1750-1833]

Fig. 1.7 Desfontaines's description of *Pistacia atlantica* in *Flora Atlantica* [3](Desfontaines, 1800) (on the left); Portrait of Rene Louiche DESFONTAINES (on the right) ((© Michael Nicholson/ CORBIS)[4]

Fig. 1.8 *Pistacia atlantica*, the tree and the Saharian Atlas in the background (El Bayadh, Algeria, March, 2014)

[3] Digitized version by © Google (accessed on 31/10/2014)
[4] http://www.magnoliabox.com/artist/7878/corbis (accessed on 8/11/215)

In his thesis, Lapie (1909) did not distinguish between the two species and called *P. atlantica* 'Terebinthe'; as well, Mathey-Dupraz (1924) cited the species as *P. atlantica* vel *terebinthus* Desf. Lapie and Maige (1924) considered *P. atlantica* as a big variety *P. terebinthus* of with a small leaves. In 1952, Zohary removed any confusion, agreed with Desfontaines (1799), De Candolle (1825) and Engler (1883); and stated *P. atlantica* as a species. Many synonyms are known for *P. atlantica* (*P. cypricola*, *Pistacia atlantica* Desf. subsp. *cypricola* H.Lindb., *Pistacia chia* Desf., *Pistacia choulettei* Gand., *Pistacia mutica* f. *multijuga* Engl…) however, *P. atlantica* is the only name considered as validly published (IPNI, 2010).

1.4 Taxonomic review of *Pistacia atlantica* complex

Morphological studies

Zohary (1952) subdivides *P. atlantica* into two subspecies (*P. atlantica* subsp. *Kurdica* and *P. atlantica* subsp. *latifolia*). He does not list *P. mutica* and *P. cabulica* which were added previously to the genus by some authors (Fischer and Meyer, 1838; Hohenacker, 1838; Engler, 1883; Marchand, 1869). He considers them as *P. atlantica* subsp. *latifolia*, while *P. mutica* has been judged as the variety of *P. terebinthus* by Nadkarni (1908).

Rechinger (1963) classifies three subspecies for *P. atlantica* in Iran: *P. atlantica* subsp. *kurdica* and the two subspecies which were not admitted by Zohary (1952) (subsp. *mutica* and subsp. *cabulica*). Yaltirik (1967a, 1967b), describing *Pistacia* species in Turkey, elevates *P. atlantica* subsp. *kurdica* from the subspecies to the species rank. He proposes a key based on presence or not of the rachis wings, fruit shape and leaflet shape (Yaltirik, 1967). Rechinger (1969) does not agree, he proposes to consider all the members of the section *Butmela* as *Pistacia atlantica* subspecies and qualified them as geographical races. He considers *P. cabulica* as *P.atlantica* subsp. *cabulica, P. mutica* as *P. atlantica* subsp. *mutica*, and *P.eurycarpa as P.atlantica* subsp. *kurdica*.

Al Yafi (1978) describes four subspecies for *P. atlantica*. He retained Rechinger's subdivision adding *P. atlantica* subsp. *atlantica* which represents the species in North Africa. He proposes his key based mainly on the presence of hairs on the leaflets, the rachis shape, the leaflet shape and the apex leaflet shape. Nevertheless, he separates again in his thesis (Al Yafi, 1979) *P. atlantica* subsp. *kurdica* and propose to consider it again, as a distinct species, he calls it *P. kurdica* (ZOH) ALYAFI comp.nov.= *P. eurycarpa* YALT = *P. atlantica* subsp. *kurdica* (ZOH) RECHINGER.

et al., 2009a). These authors propose that *P. atlantica* subsp. *mutica* could be classified as a distinct species as *P. mutica* and the *cabulica* as a subspecies of *P. mutica*. Nevertheless, previous morphological and molecular studies retained *P. mutica* as the subspecies of *P. atlantica* (Zohary, 1952; Kafkas, 2006) within the group of *Eu-Butmella* (Zohary, 1952). Arabnezhad *et al.* (2011) show that *Pistacia atlantica* and *P. mutica* were the closest species using SSR characterization, therefore *P. mutica* should be considered as a subspecies of *P. atlantica*. Several studies show that *P. eurycarpa* is synonym for *P. atlantica* subsp. *kurdica.* It is closely related to *P. atlantica and shows* a closer genetic similarity to *P. atlantica* than the other species in the genus, but should be considered distinct from *P. atlantica* (Kafkas and Perl-Treves, 2001; 2002; Karimi *et al*., 2009a; Karimi and Kafkas, 2011). In addition, the phylogenetic analysis using the SAMPL marker method permits to Karimi and Kafkas (2011) to postulate that *P. atlantica, P. atlantica* subsp. *mutica* and *P. atlantica* subsp. *cabulica* are descendents of *P. eurycarpa*.

1.5 Botanical description

Pistacia atlantica is called in arabic ‘Al bottm, or ‘El buttum al atlassi’ to distinguish it to *Pistacia terebinthus* which is also called sometimes El buttum. Many vernacular names are known in the different areas where it occurs (btom, bettam, battach, iggt, iqq, idj, or botma for an individual tree) (Burnet, 1939; Quezel and Santa, 1963). It is called by the Amazighs (berber populations of the Maghreb) tismelelt and tesemhalt (Burnet, 1939). It is called ‘Atlantik sakizi’ in Turkish, ‘Treminthos’ in Italian (Gregoriou, 2001), ‘Almácigo de Canarias’ or ‘Lengua de oveja’ in Spanish (Mansf Ency, 2001). This species is called Atlas pistachio as a common name in English.

It is great to know that the rural populations regroup *P. atlantica, P. lentiscus* and *P. terebinthus* into the same family that they name vernacularly ‘Al Khachchab’ family which means plants giving a lot of branches (El Zerey-Belaskri *et al*., 2014a), it is in fact the *Anacardiaceae* family.
Pistacia atlantica trees are strong and vigorous (Fig. 1.11), reaching 15–20 m in height. The trunk of adult trees can exceed 1 m in diameter and 6 m in circumference (according to our field work, data not shown), with a striated dark grey bark (Fig. 1.11 B) (Desfontaines, 1799; Battandier and Trabut, 1888; Monjauze, 1980; Quezel and Santa, 1963). The researcher notes that the bark is white-beige in the steppic and Southern populations of Algeria (Fig. 1.11 C).

The bark is rich in tannin and is mostly used for tan skins (Bozorgi *et al*., 2013). The trunk is mostly single (Fig. 1.11 D) (Quezel and santa, 1963) but can be branched from base (Fig. 1.11 E).

Fig. 1. 13. *Pistacia atlantica* tree providing shade and grazing (the photos showed that accessible leaves are grazed by sheep)

This species has a strong root system which allowed it to occupy arid lands. Limane *et al*. (2014) described three root architecture types for *Pistacia atlantica* subsp. *atlantica* in Algeria. Young pistachio plants produce an initial orthogeotropic root which subsequently sends out several secondary ramifications (Fig. 1.14). It may evolve in developing a shallow root network in sandy soil. In loamy or calcareous soils, the root system develops extensive deep roots which are developed to mine deeper water reserves. The deep roots protected trees against hydrous and wind erosions, very common phenomena in areas where the pistachio grows (Fig. 1.15). The researcher had the opportunity to see a tree with 30 m root length; these powerful roots are used by an old farmer to go down during maintenance of his well. *Pistacia atlantica* root system is known as the most resistant to asphyxia root as the other species of the genus (Behboodi, 2005).

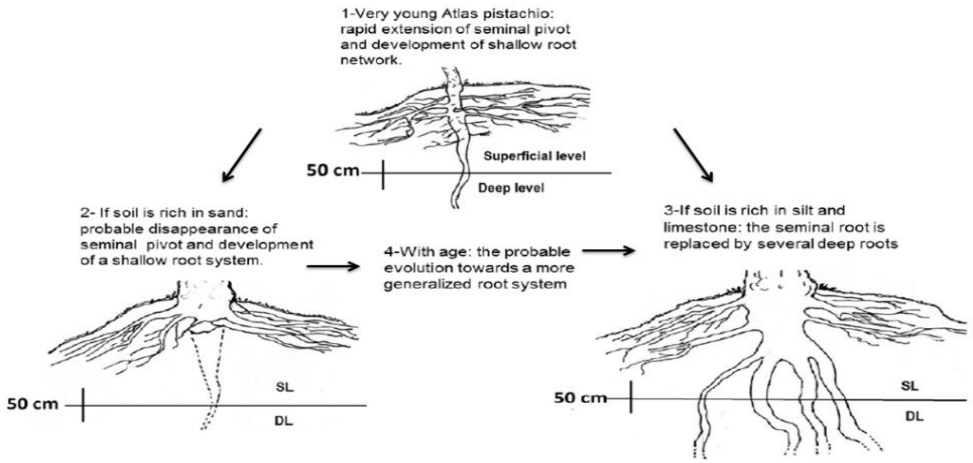


Fig. 1.14 Evolution of Atlas pistachio root system in relation to underlying soils (from, Limane *et al*. 2014)

In Anacardiaceae family, the flowers are generally not highly conspicuous but are distinctive in having an intrastaminal nectariferous disc. The flowers are almost always unisexual in Anacardiaceae and the genus *Pistacia* is characterized by its dioecious reproductive system (Mabberley, 1997).

Some cases of exceptional sex types were reported in the literature; Ozbek and Ayfer (1958) observed two hermaphrodite trees in Turkey. They reported that these trees were either seedlings of *P. vera*, or hybrids between *P. vera* and *P. terebinthus*. *Pistacia atlantica* is dioecious (Desfontaines, 1799) which showed an exceptional monoecious and/or hermaphrodite trees (Crane, 1974; Kafkas *et al*., 2001; İsfendiyaroğlu, 2007; Yaaquobi *et al*, 2009). This exceptional trait may have arisen as a somatic mutation and may be expressed as result of an interaction with an unknown biotic/abiotic environmental factor (Kafkas *et al*., 2001).

Pistacia is distinguished from other Anacardiaceae members by its reduced flower structure, plumose styles, unusual pollen morphology (Pell, 2004; Perveen and Qaiser, 2010), the absence of nectariferous disc and the petals. The two last characters explain the strict anemophilous pollination of the genus. Nevertheless, the bees may visit the male flowers to feed on pollen (Brichet, 1923). In *Pistacia atlantica* flowers are unisexual, actinomorphic and apetalous. The haplostemonous male flowers in the genus *Pistacia* supported its position within the tribe *Rhoeae* (Engler, 1876; Mitchell and Mori, 1987).

Flowering occurs before vegetative development and males tend to flower before females (Protandry) (Fig. 1.17 B, B', C, C') (Rostamikia and Akbar, 2009). Males invest more resources in flowering early in the season before leaf production (Delph, 1999). So resources allocated to the development of vegetative growth, including photosynthetic tissues (leaf) may be limited or unsteady (Inbar and Kark, 2007). The researcher observed that unlike in flower development, female trees develop leaf buds before males (Fig. 1.17 D, D', E, E', F, F', G, G').

Briefly, trees begin to bloom with the arrival of warmer weather in March; the male pollinates the females via the April winds (Zohary, 1952). The male flowers are clustered in a terminal racemous-thyrsoid disposition (Fig. 1. 17 D, D'), joined at the base and supporting yellow round-shaped pollen sacs).

1.6 Geographic distribution and ecology

Pistacia atlantica is one of the Irano-Touranian taxa which is largely distributed (Fig. 1.23) in the Mediterranean region (Zohary 1952). The Mediterranean and the Irano-Turanian regions have many common taxa because they belong to the same climate type and are in contact since ancient geological times (Eig, 1932).

Monjauze (1980) describes it as the most ubiquitous tree in North Africa and the Middle East. In Europe, the species grows in Greece (in Attica region and in the islands of Rhodos and Chios) (Rouskas, 1996), in Serbia (Al Saghir, 2006) and in Ukraine (Krym) (USDA/ARS/GRIN, Online Database). In Asia, *Pistacia atlantica* is found from Northern and Western Pakistan, to central and South Afghanistan, South and West Iran, the Southeast Caucasus, North Iraq, South Turkey, Syria, Lebanon, Jordan to Palestine.

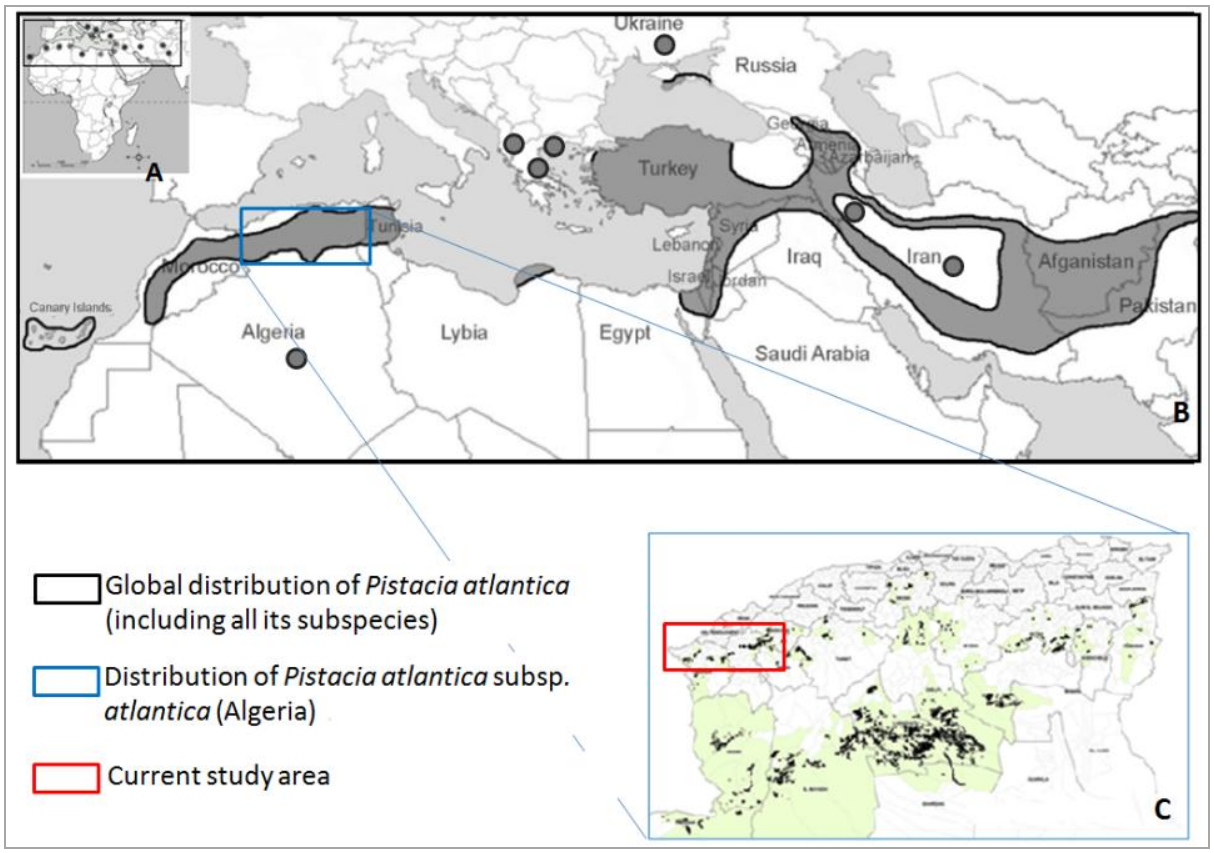


Fig. 1.23 *Pistacia atlantica* distribution map: global distribution according to Al Saghir (2006) (A), to Borowicz (1988) and Zohary (1996) (modified in (B)). Distribution map in Algeria (C) modified from DGF (2015)

In North Africa, it occurs in isolated stands in Egypt and Lybia but builds massive stands in Tunisia, Algeria and Morocco (Desfontaines, 1779; Zohary, 1952; Quezel and Santa, 1963; Monjauze, 1968; Browicz, 1988). It reapers in Canary Islands (Ceballos and Ortuño, 1976), western limit of its wide distribution area. It is believed that *P. atlantica* is originated of Persan region (Iran) from where it has expended to Southwest of Europe, to North Africa and the Canary Islands Zohary (1952). In these different geographic locations it occurs with its different subspecies (Monjauze, 1968; Behboodi, 2004).

Pistacia atlantica subsp. *cabulica* mostly grows in regions with less altitude, but it is found until 2500 m. It is distributed in regions which have less than 100 mm rain yearly, and is spread to the regions until 200 mm. This subspecies is the most resistant to the lack of water (Behboodi, 2005). This subspecies is found in Afghanistan, Pakistan, and Iran (Rechinger, 1969; Behboodi, 2004).

Pistacia atlantica subsp. *mutica* occurs in high altitude, between 900-2800 m. It grows in regions that have 200-400 mm of yearly rain; it is found in Armenia, Crimea, Turkey, Iran and the Caucus (Brichet, 1931; Rechinger, 1969; Behboodi, 2004; 2005). *Pistacia atlantica* subsp. *kurdica (=P. eurycarpa)* is also found in high altitude (900-2800 m) and is distributed in regions with 500-600 mm of rain per year. It occurs in Iran, Iraq, Syria, Turkey and Palestine (Rechinger, 1969; Behboodi, 2004; 2005).

Pistacia atlantica subsp. *atlantica* is native to the Maghreb countries (Bowicz, 1988). It is possible that this was the reason which leaded Quezel and Santa (1963) and Ozenda (1983) to describe *P. atlantica* as endemic to North Africa. However, the subspecies is cited in Syria, Lebanon and Turkey (Karimi and Kafkas, 2011; Al Saghir and Porter, 2012). This subspecies is found in limited sites in Egypt and Libya probably because of the latitude, but it occurs in more large areas in Tunisia, Algeria and Morocco. *P. atlantica* grows at altitudes ranging from 100 m (Kalaa, Tunisia) to very high elevations (2000 m, in Algeria) (Boudy, 1952; Monjauze, 1968). It is xerophilous tree, known for its exceptional drought plasticity which could be its main trait (Monjauze, 1980). It builds up park-forests (Fig. 1.24) and often grows as a dominant constituent of steppe-forest formations (Fig. 1.25) (Zohary, 1996).

Atlas pistachio occupies a wide variety of soils. It shows no soil preference and thrives well in dry and poor soils, in clay or silty soils (Boudy, 1952; Limane, 2014). It has the capacity to colonize the rocky soils (Fig. 1.26) where roots insinuate and develop inside cracks and where it can overcome many other species in growing competition (Monjauze, 1968; 1980).

Fig. 1.24 *Pistacia atlantica* in a park-forest in the region of Ain Fras, Mascara (April 2013)

Fig. 1.25 *Pistacia atlantica* trees from an old steppe-forest in the region of El Abiod Sidi Echikh, El Bayadh (March 2014)

CHAPTER 2
CLIMATIC CHARACTERIZATION OF *Pistacia atlantica* subsp. *atlantica* NATURAL AREA IN NORTHWEST ALGERIA

This chapter was discussed at the '2nd International Conference of Plant Biodiversity' Marrakech (Morocco) march 27-29, (El Zerey-Belaskri *et al*., 2014a).

2 CLIMATIC CHARACTERIZATION OF *Pistacia atlantica* subsp. *atlantica* NATURAL AREA IN NORTHWEST ALGERIA

2.0 Background

The definition of climate change is given by Intergovernmental Panel on Climate Change (IPCC) as follows: "A change in the state of the climate that can be identified (e.g., by using statistical tests) by changes in the mean and/or the variability of its properties, and that persists for an extended period, typically decades or longer. Climate change may be due to natural internal processes or external forcing or to persistent anthropogenic changes in the composition of the atmosphere or in land use" (IPCC, 2007). Climate change induces climate variability of temperature and precipitation as well as the frequency and severity of weather events. Climate change includes indirectly changes in land and water condition, soil moisture, change in frequency of fire and pest infect and the distribution of diseases. The concept of ''Mediterranean'' climate is characterized by mild wet winters and warm to hot, dry summers and may occur on the west side of continents between about 30° and 40° latitude. However, the presence of a relatively large mass of water is unique to the actual Mediterranean region (Lionello *et al*., 2006). The Mediterranean Region has many paleontological and geomorphological characteristics which make its climate scientifically interesting (Lionello *et al*., 2006).Climate variability is one of the main factors shaping the morphology, the physiology, and genetic differentiation of plants (Linhart and Grant 1996; Hufford and Mazer 2003; Jump and Penuelas 2005; De Jong 2005). It affects also the productions and prices in agriculture field (Oxfam, 2006). Bradshaw (1965) suggested that a variable environment within the life span of an organism should favor a flexible phenotype. Many investigations mainly focused on the morphological and biochemical variability on *Pistacia atlantica* were carried out in the southeast and the southwest of Algeria (Belhadj *et al*., 2007; Ait Said *et al*., 2011). Many other studies are done in other regions (High central plains of Algeria) (Mehdeb, 2011; Dahmani, 2011). The Northwestern populations have not been previously considered. In this large area, many natural populations occur under different environmental factors. Before beginning any biological investigation on the target populations, it is important to conduct a study on the climatic situation in the area. In the current study, the climatic characterization was done according to the best known methods and index for the Mediterranean climate and region (De Martonne, 1926; Emberger 1930; 1941; Debrach, 1953; Musset, 1953; Emberger 1955; Stewart, 1969). The bioclimatic synthesis carried out on the study area informs about the ecological character of *Pistacia atlantica*. In addition, it is a useful data to

The sites were prospected from fourteen natural populations : Bétaim (BTM), Chigguer (CH), Ourit (OR), Ain Fezza (AF1 + AF2), Ouled Mimoun (OM), Tallout (TA) belong to the administrative region (wilaya) of Tlemcen; Ben Badis (BB), Sidi Bel Abbes (SBA), Mustpha Ben Brahim (MBB1 + MBB2), Sfisef (SF1 + SF2), Beni Tala (BT) belong to the administrative region of Sidi Bel Abbes; Graia (GR), Bouhanifia (BH) and Trois rivières (3R) belong to the administrative region of Mascara.

2.1.2 *Climatic characterization of the study area*

To characterize bioclimatically the different sampling sites, climatic data averaged over the last 30 years was used. The basic data was provided from the National Office of Meteorology (ONM, 1981; 2005; 2011). Two capital parameters were taken into account, precipitation and temperature. The following climatic index was determined:

a/ average monthly precipitation and average annual precipitation;

b/ seasonal rainfall regime according to Musset (1953)'s coefficient; This coefficient (*Srr*= Ps*4/P) ranks the seasons in decreasing order of rainfall, where Ps is the seasonal precipitation and P is the average annual precipitation;

c/ average monthly temperature, average annual temperature;

d/ Continentality index (M-m) according to Debrach (1953): Thermal classification of the climates according to Debrach (1953) is based on the temperature range (M-m): [Insular climate: M-m < 15 °C; Littoral climate: 15 °C < M-m < 25 °C; Semi-continental climate: 25 °C < M-m < 35 °C; Continental climate: 35 °C < M-m]. Another classification is proposed by the author based on the formula (M+m/2): [Warmer climate: M+m/ 2>20 °C; Moderate climate 15 °C<M+m/2<20°C; Cold climate 10 °C<M+m/2<15 °C; Very cold Climate M+m/ 2<10 °C];

e/ Summer drought index (Emberger, 1941): This index (Is= P/M) is the ratio of the total amount of average summer precipitation to the mean of the average highest temperatures of the warmest month;

f/ Aridity index (De Martonne, 1926): This index can be calculated for yearly, half yearly, seasonal or monthly reference periods. Lower values indicate drier areas. In our analysis the aridity index was calculated as yearly values according to De Martonne's method (De Martonne 1926). The Aridity Index (I^{DM} = P/T+10) is the ratio of the average annual precipitation to (the average annual temperature to which the constant 10 is added). The bioclimatic type is described according to a scale [Hyper arid: $0<I^{DM}<5$; Arid: $5< I^{DM} <10$; Semi-arid: $10< I^{DM} <20$; Sub-humid: $20< I^{DM} <30$; Humid: $30< I^{DM} <55$; Perhumid: $55< I^{DM}$];

g/ Pluviothermal quotient according to Stewart (1969): Based on Emberger's pluviothermal quotient '*Q2*' (Emberger, 1930; 1955), Stewart (1969) has established for the Algerian bioclimate

Average monthly precipitation and average annual precipitation

Among the sampling sites, AF and OR are the rainiest with respectively 537.36 mm and 501.48 mm of total precipitation average. The sites BB and MBB are in the second position with respectively 388.33 mm and 378.7 mm (Table 2.1). January and March are the rainiest months in AF, OR, TA and BB. The two first sites record the most important rainfalls. March and November are the rainiest in BTM. For the rest of the locations, January and November are the rainiest months (Fig. 2.2).

Seasonal rainfall regime according to Musset (1953)'s coefficient

Two courses are observed in the study area (Fig. 2.3). Among the harvested sites, BTM, CH, OR, AF, TA, BH and 3R follow the course as WSASu, which means that winter and spring are the rainiest seasons. The second group contains the other sites SBA, MBB, SF, BT, and GR which follow the course as WASSu, where winter is the rainiest season.

Average monthly temperature and average annual temperature

In the current study area, the average annual temperature ranges between 16.51°C and 21.86°C (Table 2.1). January is the coldest month (Fig. 2.4) with average temperature raging between 8.91 to 14.58 °C. July is the warmest month in OM and TA with respectively 26.01°C and 26.63°C. For the other sites, August is the warmest month recording 26.34 °C to 32.35°C. The maximum temperature of warmest month ranges between 32.98°C and 41.43, while the minimum temperature of coldest month varies between 3.11°C and 6.41°C.

Continentality index (M-m) (Debrach, 1953)

Referring to (M-m), 9 sites are under 'Semi-continental' climate, while 5 sites are under 'continental climate. Referring to (M+m/2), 6 sites are under moderate climate while 8 are under a warmer climate. Taking into account the two classifications, we obtain: BTM, CH, SBA, SF, BT and GR under a Moderate semi-continental climate; OR, AF and MBB under a warmer Semi-continental climate; and OM, TA, BB, BH and 3R under warmer continental climate (Table 2.1).

Several climatic index were developed by combining usually data of rainfall, evaporation, and temperature. The use of bioclimatic index could be a major step forward in the methodology of ecological studies. Three index were investigated in the current study to characterize the different sites where occur the natural populations of *Pistacia atlantica* subsp*. atlantica.*

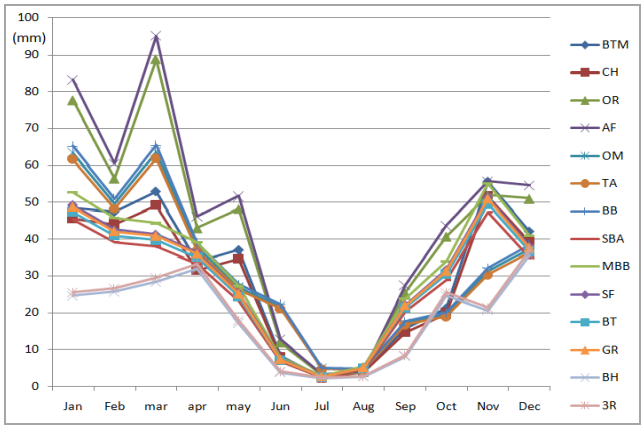


Fig. 2.2 Average monthly precipitation in the different study sites (1980-2010)

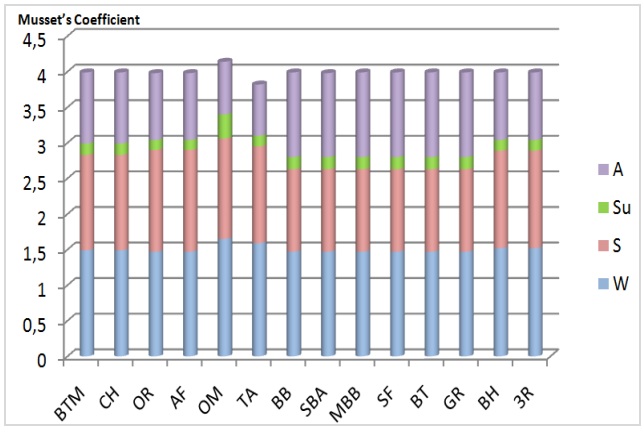


Fig. 2.3 Seasonal rainfall distribution according to Musset (1953)'s coefficient (1980-2010)

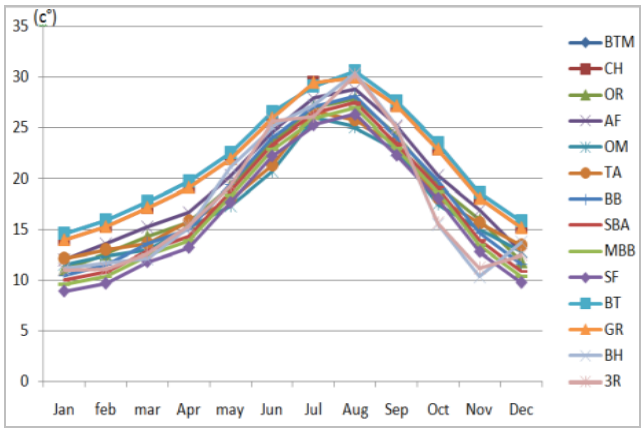


Fig. 2.4 Average monthly temperature in the different study sites (1980-2010)

Summer drought index (SDI), Aridity index (dMI) and Pluviothermal quotient (Q_3)

The summer drought index calculated for all the sites is below 1 (table 2.1). Low values of this index indicate an important summer drought, with scarce rains and high temperatures. The sites OR and AF have recorded a drought index higher than 30. They are under a humid climate according to De Martonne's index. Except the sites BH and 3R which are under arid climate, the other sites are under semi-arid climate. Among the different sites of the study area, CH, OR, AF, OM, TA, SBA and GR are under Semi-arid with temperate winter bioclimate according the Emberger's pluviothermal quotient. The sites BTM, MBB, SF, BT, BH and 3R are under Arid with temperate winter.

2.2.2 *Classification of sampling sites in bioclimatic homogenous areas*

At the end of this characterization, the bioclimatic data submitted to the Ascendant Hierarchical Classification defines five groups of bioclimatically homogeneous area; (Fig. 2.5). BTM group contains (BTM, CH and GR) according to the altitude; OM group contains (OM, TA, BB, and MBB) according to altitude, climate type (*sensu* De Martonne) and temperature range; SBA group with (SBA, SF, BT) according to climate type (*sensu* De Martonne) and temperature range; BH group containing (BH and 3R) according to bioclimatic stage and temperature range; and OR group containing (OR and AF) according to altitude, climate type (*sensu* De Martonne), temperature range and bioclimatic stage.

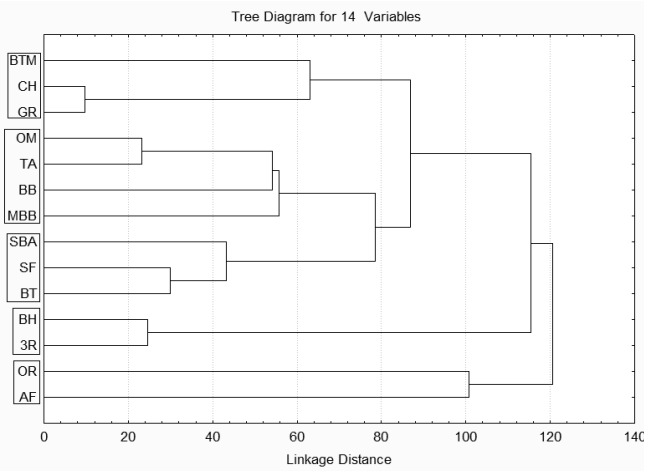


Fig. 2.5 Classification of the sampling sites in bioclimatic homogenous areas (obtained by two dimensional dendrogram; Horizontal: similarity levels between sites. Vertical: investigated sites

2.3 Discussion

The study area is under Mediterranean climate (Emberger 1942; 1955; Seltzer 1946; Angot 1981), and the climate is the first characteristic of the Mediterranean ecosystem (Daget 1977; Aidoud 1989). One of the most essential purposes of the Mediterranean ecology is to check out the relation between the plant formations and the climate, seen from a bioclimatic perspective. The climatic characterization of the studied sites still indicates the ecological plasticity of this species. It shows that *Pistacia atlantica* occurs in North Algeria under different climatic conditions. Rainfall in the study area ranges from 225 mm/y to 537.36 mm/y. The populations of AF and OR

are located on Tlemcen Mountains, one of the rainiest regions in Northwest Algeria (Meddi and Hubert 2002).

In fact, this species grows in a wide range of rainfall. Monjauze (1968) found it in Algeria between 68 mm and 1200 mm of annual rainfall. In the region of Bechar (South-west of Algeria), Atlas pistachio populations are under 58 mm (Ait Said *et al*,. 2011), while in Hoggar mountains, some specimens survive with 20 mm to 48 mm of rainfall (Monjauze 1968; Kadi-Benane 2004). Boudy (1952) states that 150 mm to 250 mm of rain is sufficient for this species. In Tunisia, it occurs under 150 mm to 480 mm (Ghorbel *et al*., 1998), and in Lebanon, it grows under 400 mm to 600 mm of rain (Talhouk *et al*,. 1998).

According to Musset's Seasonal Rainfall Regime, the study area is under two groups of regime. The first type (WSASu) is a 'classical' rainfall regime in Algeria where winter is the rainiest season (Daget, 1980; Benabadji and Bouazza, 2000; Meddour 2010). In the Northwestern area of Algeria, particulary in the region of Tlemcen, winter and spring are the rainiest seasons (Aboura, 2006). The second type (WASSu) is also very common in Northwest Algeria. Summer is in all the sites the driest season. In fact, this is one of the characters of Mediterranean climate. The seasonal rainfall is an important data for studying plant water demands. In the current study area, the lower temperature is recorded in January (8.91 °C) and the highest one in august (30.58°C). It is reported that Atlas pistachio supports more than 43 °C as maximum temperature and 0.3 °C as minimum temperature (Monjauze, 1968). It may survive in lower temperatures mainly in steppe and desert.

The thermal amplitude measured according to Debrach's index indicates the continentality of the study area. In North Africa, the declining summer precipitation should lead to the accentuation of the phenomena of continentality, which induce the steppisation or the desertification (Medail and Quezel 2003). The summer drought index (Emberger, 1942), calculated for the different sites show a marked Summer drought which is a discriminate character for the mediterraneity (Daget, 1977). However, even under those conditions, the species of this area are well adapted to the drought (besancenot, 1986). Alcaraz (1969) notes that in West Algeria, many species are adapted to low index (less than 2). In Mediterranean countries, *P. atlantica* colonizes regions with dry and a pronounced hot season (monjauze, 1980). De Martonne's Aridity index (De Martonne, 1926) was successfully used in different species distribution studies (Rol, 1937; Claessens and Thibaut, 1995; Laaidi, 1997; Charnay, 2001).

Our results show that *Pistacia atlantica* is well widespread in the area where Aridity index is comprised between 20 and 30, and it can reach humid areas where Aridity index is comprised between 30 and 55.

According to Emberger (1955), the Mediterranean climate is drier when the quotient is small. In the current study area, Atlas pistachio occurs under three bioclimatic ranges (*sensu* Emberger), from the arid to the sub-humid. This distribution was always noted for this species known for its plasticity and its distinctive xerophytic character (Boudy, 1950; Monjauze 1980; Zohary 1996). In addition to the climatic plasticity, the altitudinal range of *P. atlantica* is very wide. In this study, Atlas pistachio populations are observed between 305 to 821 m. In Algeria and Morocco, it is found from 500 m to 2000 (Monjauze, 1980; Chenoune, 2005; Benhassaini *et al*., 2007b). It is observed in Tunisia from 100 m to 2000 m (Ghorbel *et al*., 1998). In its Asian area, it is cited between 300 and 1800 m in Turkey, between 900 and 3000 m in Iran, between 600 and 1800 m in Iraq, between 900 and 1400 m in Pakistan, between 850 and 2500 m in Afghanistan and between -50 and 1200 m in Palestine (Zohary, 1996; Behboodi, 2005).

2.4 Conclusion

The climatic characterization in this chapter showed that Atlas pistachio populations are under different climatic conditions ranging from humid to arid climate (*sensu* De Martonne) and from sub-humid to arid bioclimate (*sensu* Emberger). Annual precipitation, average annual temperature and elevation varied between 225 and 537.36 mm, between 16.51 T°C and 21.86 T°C, and from 305 to 821 m respectively. The results of this chapter refer to the ecological plasticity of Atlas pistachio.

CHAPTER 3
MORPHOLOGICAL CHARACTERIZATION AND MORPHOLOGICAL VARIABILITY ANALYSIS OF *Pistacia atlantica* subsp. *atlantica* IN NORTHWEST ALGERIA

Part of this chapter is published as 'El Zerey-Belaskri A. and Benhassaini H. 2015. Morphological leaf variability in natural populations of *Pistacia atlantica* Desf.subsp. *atlantica* along climatic gradient: new features to update *Pistacia atlantica* subsp. *atlantica* key. *International Journal of Biometeorology*, doi 10.1007/s00484-015-1052-4.

3 MORPHOLOGICAL CHARACTERIZATION AND MORPHOLOGICAL VARIABILITY ANALYSIS OF *Pistacia atlantica* subsp. *atlantica* IN NORTHWEST ALGERIA

3.0 Background

As seen previously, *Pistacia atlantica* is known for its distinctive ecological plasticity (Boudy, 1950; Monjauze, 1980; Zohary, 1996; Ait Said *et al*., 2011; El Zerey-Belaskri *et al*., 2014a). Both ecological plasticity and phenotypic plasticity are closely related (West-Eberhard, 1989). This latter concept is defined as the variability of phenotypic expression of one genotype and it is measured by its 'Reaction Norm' (Agrawal, 2001). The reaction norm is defined as the phenotype range produced by one genotype when exposed to different environments (Trevor *et al*., 2003). It is likely that phenotypic plasticity plays an important role in diversification (West-Eberhard, 1989). This plasticity is expressed by different levels of morphological variability which give to the species phenotypic characteristics. These characteristics are an unavoidable tool for the classification and the organism taxonomy. They serve also to recognize the hybrids or the contaminations (in the case of the microorganisms). The morphological features are very important for the species characterization. They have a mono or a polygenic determinism but they can be influenced by the environmental factors. When De Candolle (1868) conceived the notion of 'the characteristic leaf', he means that the species is not characterized only by the reproductive traits in flower and fruit on which the Linneaus system was based; but also by vegetative traits. Indeed, in the genus *Pistacia*, the diagnostic traits used by Zohary (1952, 1972) to distinguish between the various species are mainly leaf characteristics and nut morphology since *Pistacia* is characterized by its homeochlamydic perianth (Mabberley, 1997) and reduced flower structure (e.g. naked flowers) (Pell, 2004).

The morphological features are determined by the biometric measurements (organs size and shape) and qualitative data (organs color, texture and smell). They are selected and standardized as descriptors (Painting *et al*., 1993). Plant Descriptor is an identifiable and quantifiable characteristic (mainly botanic and taxonomic features) of one species used in the characterization of the populations aiming to distinguish, classify, store and recover the data (Painting *et al*., 1993). The International Plant Genetic Resources Institute (IPGRI) has published descriptor lists, which provide detailed information about how to collect phenotypic data for the characterization of *Pistacia* species (IPGRI, 1997; 1998). The first report (IPGRI, 1997) describes *Pistacia vera* while the other species are standardized in IPGRI (1998).

Purposes

The objective of the morphological study is to characterize the morphological diversity in natural populations of *Pistacia atlantica* subsp. *atlantica* in Northwest Algeria; to analyze the morphological variability within and between individuals and populations; to update the morphometric data of *Pistacia atlantica* subsp. *atlantica.* Finally, the effect of climatic variation on the observed morphological variability is to check.

3.1 Material and methods

3.1.1 *Plant material*

The quantified description of species leaf shape faces many biological and physiological constraints. According to Mouton (1967), the typical leaf of a given species is the one having completed its growth. That is why the sampling, in the current study, was conducted in the period October to December (2010 to 2013). The foliar measurements were performed on mature leaves, median and terminal leaflets excluding (young leaves, leaves developing from adventitious buds and infested leaves). Six vigorous trees per site were selected (3 male and 3 female trees). From each tree and from each aspect (North, south, west, east) ten (10) leaves were collected and were put in labeled plastic bags, totaling 40 leaves per tree. For the tree description, more trees are taking into account (5 to 10 trees are described per site).

Tree descriptors

The measurement and the description have been done according to (IPGRI, 1998). *Pistacia atlantica* trees were described according to three criteria:

a. **Tree vigour**: Low, Intermediate or High;

b. **Growth habit**: Tree with single trunk, Tree much branched form base;

c. **Branching habit**: Sparse, Intermediate, Dense.

Leaf descriptors

Once in the laboratory, on 3520 leaves, 15 leaf characters (10 quantitative and 5 qualitative) are described according to IGPRI (1998). The measurement were done, using ‘a precision caliper (0,01mm)’, leaf length and width, petiole and rachis length, terminal leaflet length and width, terminal leaflet’s petiolule length, and terminal and median leaflets thickness (Fig. 3.1). In addition, the number of leaflets was scored. The leaflet shape, the median and terminal leaflet apex shapes and the median and terminal base shapes were described according to (IGPRI, 1998;

Judd *et al*., 2002). The leaflet color was defined according three descriptors (light green, dark green, very dark green) (IGPRI, 1998). Hand drawings were performed.

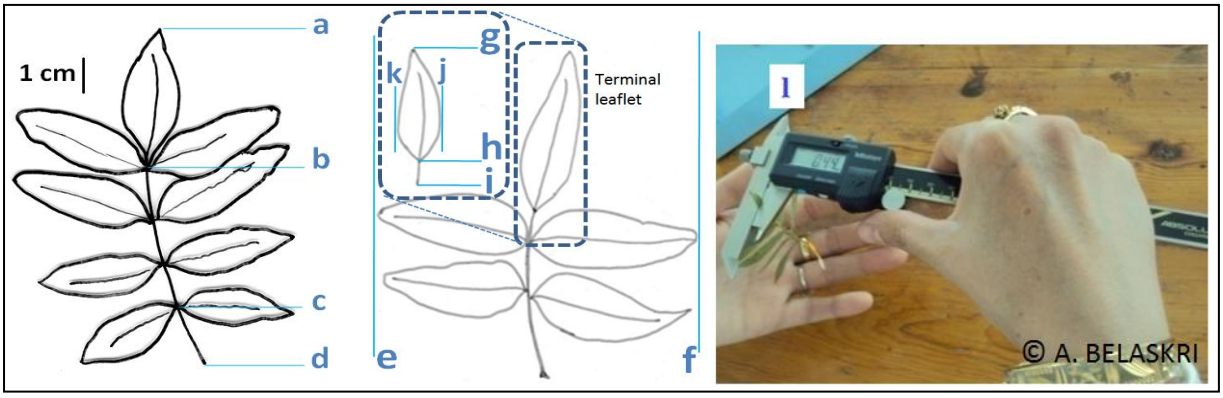


Fig. 3.1 Diagram of leaf measurement: [a-d] leaf length, [c-d] petiole length, [b-c]rachis length, [e-f] leaf width, [g-i] terminal leaflet length, [h-i] terminal petiolule length, [k-j]terminal leaflet width, [l] thickness measurment.

3.1.2 *Statistical analysis*

The data obtained in this study is submitted to a multivariate statistical analysis using Statistica software (Copyright© Stat Soft.Inc. 1984-2008).

- The morphometric data is recorded, processed by a nested ANOVA and classified by a hierarchical clustering (HAC) using Ward linkage with square Euclidean distance.
- Aiming to check the correlation between the morphological variability and the climate factors the correlation coefficient (*co*) was calculated. The clusters performed on the climatic and the morphological data were compared.

3.2 Results

3.2.1 *Habitus characterization*

Pistacia atlantica trees (Fig. 3.2), observed in the study area, are mostly robust. Vigorous trees are observed in all the sites, however, intermediate trees are observed in three sites (18.75%) (Table 3.1). Referring to growth habit and branching habit, most of the trees have a single trunk, while some individuals are branched from base in four sites (character frequency: 25%). Atlas pistachio trees in the current study show dense foliage in all the sites. All the sites contain male and female trees.

Fig. 3.2 *Pistacia atlantica* tree description: (a) high vigour, individual trunk and dense foliage (May, 2013); (b) intermediate vigour, individual trunk (September, 2011); (c) intermediate vigour, branched from base (April, 2013).

Table 3.1 *Pistacia atlantica* tree description in the different studied sites according to IPGRI descriptors (IPGRI, 1998)

Sites	Tree sex	Vigour	Growth habit	Branching habit
BTM	Male & female	High	Single trunk	-Dense
CH	Male & female	High	Single trunk, Branched from base	-Dense
OR	Male & female	High	Single trunk	-Dense
AF	Male & female	High	Single trunk, Branched from base	-Dense
OM	Male & female	High	Single trunk	-Dense
TA	Male & female	High	Single trunk	-Dense
BB	Male & female	High	Single trunk	-Dense
SBA	Male & female	High	Single trunk	-Dense
MBB1	Male & female	High	Single trunk	-Dense
MBB2	Male & female	High	Single trunk	-Dense
SF1	Male & female	High	Single trunk	-Dense
SF2	Male & female	High, Intermediate	Single trunk, Branched from base	-Dense
BT	Male & female	High, Intermediate	Single trunk	-Dense
GR	Male & female	High	Single trunk, Branched from base	-Dense
BH	Male & female	High, Intermediate	Single trunk	-Dense
3R	Male & female	High	Single trunk	-Dense

3.2.2 *Morphological and morphometric leaf characterization*

Morphometric analysis

The morphometric leaf characterization in the different sites show significant inter and intra populational variability.

The mean leaf length and wide are variable (Fig. 3.3). The mean leaf length varies between 9.1±1.7 cm and 14.23±1.8 cm. The longest leaves are observed in AF (24.5 cm), SF1 (20.3 cm), BT (19.95 cm) and OR (18.3 cm), while the smallest leaf measures 3.4 cm (in CH). The mean leaf width varies between 6.68±1 cm and 11±1.4 cm. The largest leaves are observed in AF (21.9 cm), BT (15.9 cm) and OM and MBB2 (15.8 cm), while the less large leaf measures 1.6 cm (in OM).

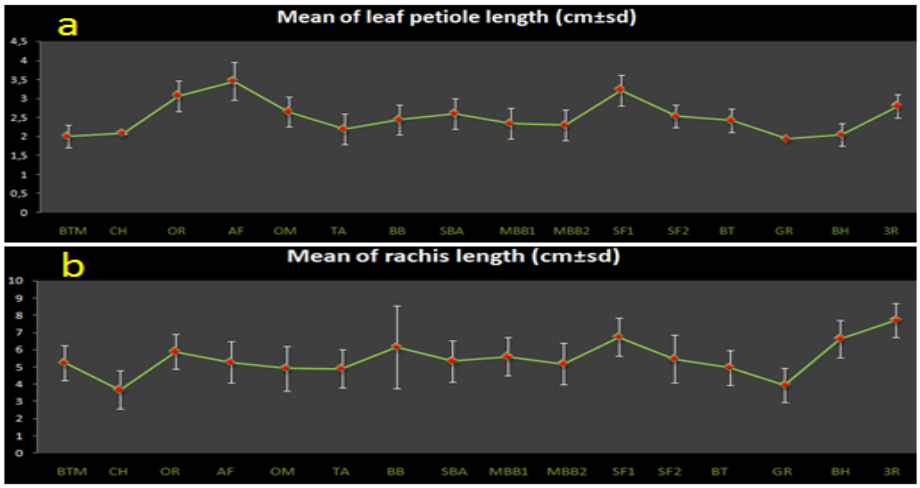


Fig. 3.5 Mean variation of leaf petiole length (a) and rachis length (b) in *Pistacia atlantica* subsp. *atlantica*

The terminal leaflet measures are variable between the sites (up to 13.6 cm long and 4.3 cm wide (in AF). The mean of the terminal leaflet length varies (Fig. 3.6) from 5.15±1.1 cm to 3.3±1.1 cm, while the mean of the terminal leaflet width varies from 1.83±0.2 cm to 0.2±0.03 cm. The terminal leaflet may be sessile or petiolulated (see Fig. 3.4). In this case, the terminal leaflet petiole attains a length of 3.4 cm (in MBB1). The leaf length is positively correlated to the terminal leaflet length (r= +0.8).

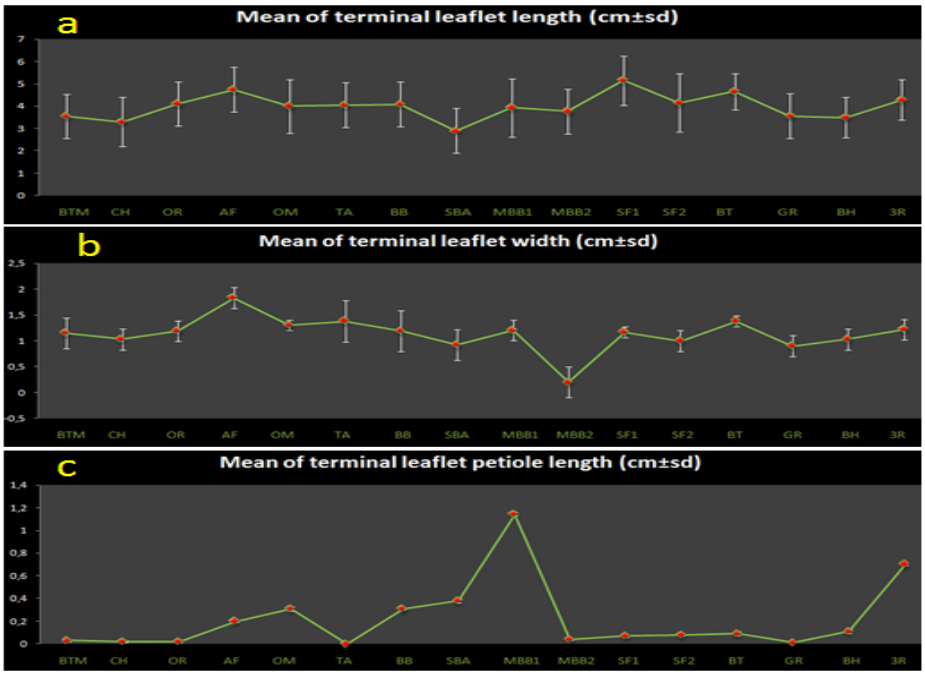


Fig. 3.6 Mean variation of terminal leaflet length (a); terminal leaflet width (b) and terminal leaflet petiole length (c) in *Pistacia atlantica* subsp. *atlantica*

Table 3.3 ANOVA table for leaf measurement *(P<0.05)*

	LEAF LENGTH (LL)					LEAF WIDTH (LW)					LEAF PETIOL LENGTH (LPL)					RACHIS LENGTH (RL)				
	SS	df	MS	F	P	SS	df	MS	F	P	SS	df	MS	F	P	SS	df	MS	F	P
Site	448.9	15	299.3	56.4	0.000000	4114.6	15	274.3	68.4	0.000000	435.9	15	29	106.5	0.000000	16777.4	15	1118.4	576.7	0.000000
Tree (site)	313	5	62.7	11.8	0.000000	231.4	5	46.2	11.5	0.000000	18.1	5	3.6	13.3	0.000000	101.8	5	20.3	10.5	0.000000
Sex (tree)	2.9	1	2.8	0.5	0.46	0.05	1	0.0458	0.01	0.91	0.0488	1	0.04	0.1	0.67	0.44	1	0.439	0.2263	0.63
Aspect (tree)	15.6	3	5.207	0.9	0.58	45.69	3	15.2303	3.8	0.02	1.2263	3	0.4	1.4	0.16	3.83	3	1.275	0.6576	0.88
Total	780.4	24				4391.74	24				455.2751	24				16883.47	24			

	TERMINAL LEAFLET LENGTH (TlfL)					TERMINAL LEAFLET PETIOLE LENGTH (TlfpL)					TERMINAL LEAFLET WIDTH (TlfW)					NUMBER of LEAFLETS (Nlf)				
	SS	df	MS	F	P	SS	df	MS	F	P	SS	df	MS	F	P	SS	df	MS	F	P
Site	965.7	15	64.3	21.2	0.000000	56	15	3.7	30.6	0.000000	282.6	15	18.8	27.8	0.000000	1462.9	15	97.5	23.7	0.000000
Tree (site)	35.7	5	7.1	2.3	0.086	1.1	5	0.2	1.8	0.153	5.4	5	1.1	1.6	0.000000	220.7	5	44.1	10.7	0.000000
Sex (tree)	2.2	1	2.2	0.7	0.3	0.4	1	0.4	3.4	0.01	0.2	1	0.2	0.3	0.5	0.1	1	0.1	0.02	0.8
Aspect (tree)	6.9	3	2.3	0.7	0.74	0.3	3	0.2	0.8	0.31	2.9	3	0.9	1.4	0.27	37.8	3	12.6	3.07	0.02
Total	1010.5	24				57.8	24				291.1	24				1721.5	24			

Leaflet thickness

The thickness measurement (Table 3.4) shows that the mean of the median leaflet thickness ranges from 0.16±0.4 mm (in SF1) to 0.49±0.3 mm (in CH). The maximal value of median leaflets thickness (0.67 mm) is recorded in the site BB. The median leaflet thickness shows high values in OM (0.57 mm), SF1 and SF2 (0.56 mm). The mean of the terminal leaflet thickness varies between 0.17±0.3 mm (in OR) to 0.5±0.4 mm (in SF2). The maximal value is noted in SF2 (0.61 mm) and in OM (0.59 mm). ANOVA analysis for the median and terminal leaflet thickness shows highly significant difference (P<0.001) between the investigated sites (Sum of squares (SS=0.33); P=1.7597E-09). However, no significant difference has been shown between the two characters within site (Sum of squares (SS=0.003); P=0.26). A weak negative correlation is recorded between the leaf ratio (Length/width) and the terminal leaflet thickness (r= -0.05) and the median leaflet thickness (r= -0.22).

Table 3.4 Thickness measurement of Terminal leaflet and median leaflet of *P. atlantica* subsp. *atlantica* (Me, M, m: mean, maximum, m: minimum value respectively)

	TERMILAL LEAFLET THICKNESS (mm)			MEDIAN LEAFLET THICKNESS (mm)		
	Me	M	m	Me	M	m
BTM	0.24±0.07	0.35	0.09	0.23±0.06	0.31	0.15
CH	0.19±0.04	0.29	0.11	0.16±0.04	0.24	0.09
OR	0.17±0.03	0.23	0.13	0.18±0.03	0.21	0.11
AF	0.29±0.03	0.34	0.18	0.3±0.02	0.39	0.24
OM	0.46±0.06	**0.59**	0.39	0.46±0.06	**0.57**	0.37
TA	0.26±0.02	0.35	0.15	0.24±0.02	0.34	0.18
BB	0.35±0.08	0.47	0.23	0.39±0.06	**0.67**	0.24
SBA	0.38±0.04	0.53	0.23	0.39±0.02	0.45	0.29
MBB1	0.41±0.03	0.48	0.3	0.41±0.06	0.51	0.2
MBB2	0.42±0.01	0.46	0.38	0.42±0.02	0.47	0.36
SF1	0.47±0.03	0.53	0.35	**0.49**±0.03	**0.56**	0.41
SF2	**0.5**±0.04	**0.61**	0.41	**0.47**±0.04	**0.56**	0.39
BT	0.21±0.02	0.31	0.15	0.2±0.02	0.26	0.11
GR	0.24±0.03	0.32	0.17	0.23±0.03	0.28	0.16
BH	0.34±0.03	0.44	0.26	0.32±0.02	0.4	0.28
3R	0.35±0.05	0.42	0.25	0.34±0.03	0.43	0.29

3.2.3 *Correlation between ecological factors and morphological characters*

The morphological data is classified by a hierarchical clustering (Fig. 3.15). This analysis retained three main groups. The cluster I contains the sites BTM, CH, GR and BH which are characterized by high number of leaflets (14 to 18). The cluster II corresponds to the sites with a high leaf length, leaf width and petiole length (SF1, AF, OR). The rest of sites are retained in cluster III where no particular character dominates, nevertheless, the variability is clearly observed by the clustering at different distances. Taking into account the five bioclimatic homogenous groups obtained by the Ascendant Hierarchical Classification applied to the climatic conditions of the investigated sites (Fig. 2.5; see Chapter 2,), the correlation between the morphological characteristics and the climatic factors is checked. The leaf length shows a strong correlation (*r= 0.87*) with the altitude and with the annual precipitation (*r= 0.7*). The leaf width shows a strong correlation with the annual precipitation (*r= 0.79*) and the altitude (*r= 0.95*), and a moderate correlation (*r= 0.6*) with mean minimum temperature of the coldest month (m°C).The terminal leaflet length is moderately correlated to the annual precipitation (*r=0.6*) and strongly correlated to the altitude (*r= 0.81*). The leaflet number is negatively correlated to the altitude (*r= 0.63).* A moderate to strong negative correlation is found between terminal leaflet thickness and the temperature (*r= -0.63*), while a strong negative correlation is recorded between terminal leaflet thickness and the mean minimum temperature (*r= -0.65*). The median leaflet thickness is negatively strongly correlated (*r*= -0.7) to the temperature.

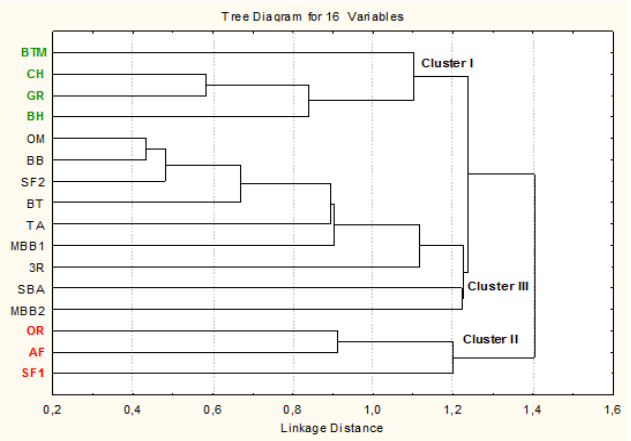


Fig. 3.15 Two dimensional dendrogram obtained in the hierarchical cluster analysis of the morphometric data of *P. atlantica* subsp. *atlantica* leaves. Horizontal: similarity levels between sites. Vertical: investigated sites

Comparing between the two dendrograms (Fig. 2.5; see Chapter 2) and (Fig. 3.15), we note that in the hierarchical classification illustrated in (Fig. 2.5), BTM, CH and GR are retained in the same group according to the annual precipitation, mean temperature and altitude. These sites are also retained in the same group by the hierarchical classification illustrated in (Fig. 3.15) according to the maximum leaflet number and the terminal leaflet length. While OR and AF are retained in the same group in (Fig. 2.5) according to the most important annual precipitation and the high altitude

In this respect, taking into account the keys proposed by Al Yafi (1978) and the Al-Saghir and Porter (2012), and responding to the findings of the current study, we propose to update the determination key of *Pistacia atlatica* Desf.subsp. *atlantica* as follows:

***Pistacia atlantica* Desf.**

Leaves large, imparipinnate, deciduous, leaf rachis winged.

1-Leaves imparipinnate 1.4 – **24.5** cm long, 1.6 – **21.9** cm wide, sometimes **paripinnate by losing the terminal or the preterminal leaflet.** Leaflets (1–) 2–**8 (−9**) pairs lanceolate, oval, elliptic, oblong, rhomboid, **obovate**, (**falciform**); obtuse, acute, acuminate, **mucronated**, **emarginate**, **rounded**, **retuse**, and **attenuate** apex leaflet. **The terminal leaflet** is sessile **or petiolulated (0.1 – 3.4 cm long)**.....subsp. *atlantica*.

(El Zerey-Belaskri and Benhassaini, 2015)

In bold: the updated features

3.3 Discussion

3.3.1 *Morphological characterization*

The current study supports that *Pistacia atlantica* subsp. *atlantica* trees are characterized by their vigour, silhouette, vigorous trunks and their dense foliage.

The old descriptions of *P. atlantica* were given by many botanists who explored North Africa and the Middle East. Desfontaine (1799), the first who described the species in his '*Flora atlantica'*, mentioned an odd-pinnate leaves with about 7 to 9 leaflets. Zohary (1952) has cited the species within the section *Butmela*, characterized by deciduous odd-pinnate leaves, composed by 2-5 pairs. Monjauze (1968) accorded with Zohary (1952) mentioning 11 leaflets. Al Yafi (1978) describing a key for the four subspecies (*atlantica*, *cabulica*, *kurdica*, and *mutica*) mentioned as maximum 5 pairs of leaflets for both of them. Recently, the key proposed by Al-Saghir and Porter (2012) for the genus *Pistacia* gave the same maximum number of leaflets (11). In Algeria, more leaflet number was observed; Belhadj *et al*. (2008) recorded 7 pairs. In this study, *P. atlantica* subsp. *atlantica* developed in BTM 18 leaflets (9 pairs), a number which has been never observed before on *P. atlantica*.

In the key proposed by Al-Saghir and Porter (2012), the genus *Pistacia* is devised into two groups, small leaves and large leaves including *P. atlantica*. The measurements recorded in this study (Table 3.2) exceed significantly those described in the previous keys (Desfontaines, 1799; Quezel and Santa, 1963; Al Yafi, 1978; Al-Saghir and Porter, 2012) and in the studies done on the morphological variability of the species and mainly of the subspecies *P. atlantica* subsp. *atlantica* in different countries (Belhdj *et al*., 2008; Karimi *et al*., 2009; Yaaqobi *et al*., 2009; Ait said *et al*., 2011; Benabdallah, 2012; Louzabi *et al*., 2014; Benaradj *et al*., 2014).

In the key proposed by Al Yafi (1978), the terminal leaflet is sessile in *P. atlantica* but subsessile with a petiolule in *P. atlantica* subsp. *kurdica,* in the current study, cases of petiolulated terminal leaflet were observed and the petiolule was about 0.1 – 3.4 cm long. *Pistacia atlantica* leaves are imparipinnate (Desfontaine, 1800; Zohary, 1952), however paripinnate leaves were observed (13.33%) in the current study because of the absence of terminal or preterminal leaves. similar results were recorded by Inbar and Kark (2007). In addition to this finding, the leaflets were alternate on the rachis in many cases. The two phenomena were observed by Inbar and Kark (2007). According to Graham *et al*. (1993) and Inbar and Kark (2007) these phenomena may be considered as indicator of developmental instability. Inbar and Kark (2007) did not record any correlation between the terminal leaflet absence and the asymmetry, neither between the phenomena and the environmental conditions or the biotic stress.

The lanceolate, oval, elliptic, oblong and rhomboid shapes were already described (Quezel and Santa, 1963; Monjauze, 1968; Al Yafi, 1978; Belhadj *et al*., 2008). However, the two shapes obovate and falciform have never been mentioned before in *P. atlantica* subsp. *atlantica.* The terminal leaflet apex is an important differential character within and between *Pistacia* species (Zohary 1952). Eight apex shapes were observed, in the current study, on *P. atlantica* leaflets. Some of them (obtuse, acute, and acuminate) were previously described (Engler, 1883; Quezel and Santa, 1963; Kafkas *et al*., 2002; Belhadj *et al*., 2008). In their keys, Engler (1883) and Al Yafi (1978) stated that *P. atlantica* did not show a mucronated apex leaflet. Nevertheless, Kafkas *et al*. (2002) have observed this shape on the species. The results of the current study are consistent with the last author, the mucronated apex was observed in two sites (OM and BH). The other shapes (emarginate, rounded, retuse and attenuate) were not previously observed. The leaflet base has not been described in detail; Ghorbel *et al*. (1998) noted the rounded shape; while six shapes (cuneate, attenuate, obtuse, rounded, deccurent, oblique) are observed in the current study. The updated Key in the current study brings supplementary data for the morphological

identification of the species *P. atlantica* in general and for the subspecies *P. atlantica* subsp. *atlantica* specifically.

Leaf colour is one of the most useful characters to take into account as descriptors for *Pistacia* species classification (Kafkas *et al*., 2002). The major cause of leaf colour change involves both external and internal factors. In the current study, the leaf colour was investigated in the different sites. It appears that *Pistacia atlantica* subsp. *atlantica* leaves are in four green colour degrees (Light green, dark green, very dark green and military green). The three first degrees were already observed in this species. In five sites a high percentage of dark green was observed; while in four sites, the frequency of 'very dark green' leaves was the highest, in agreement with Kafkas *et al*. (2002) who stated that *Pistacia atlantica* has the darkest leaves comparing to the other species (*P. eurycarpa*, *P. terebinthus*, and *P. khinjuk*). Belhadj *et al*. (2008) noted that the leaves in this species are mostly green while in 2 sites, they are darker than the other. In the present study, the light green leaves have been recorded in a high percentage in 6 sites; nonetheless, this feature is not very common in *Pistacia atlantica*. Belhadj *et al*. (2008) have observed this colour in 1/8 site and with a low percentage (7.8%). The military green was observed exclusively in one genotype, (coded as PaaAF23FG). This genotype is characterized also by long and large leaves.

In general, a specific character appears when hybridization occurs between two *Pistacia* species (Kafkas *et al*., 2002); however, in our case, no other species was found in the site. Yet, in the current study, other leaves showed resemblance in shape to the subspecies (subsp. *cabulica* and subsp. *kurdica* (= *P. euryparpa*). In fact, Zohary (1952) reported that *P. atlantica* subsp. *atlantica* and *P. atlantica* subsp. *latifolia* (synonym *P. atlantica* subsp. *mutica* and *cabulica*) are merely derivatives of *P. atlantica* subsp. *kurdica* (=*P. eurycarpa*). Leaf resemblance was also observed between some study's samples and *P. vera* and *P. khinjuk*. The appearance of this feature in *Pistacia atlantica* leaves may be due to the close relationships between this species and (*P. vera, P. khinjuk and P. eurycarpa*) which are considered as the most primitives, from which the other species desended (Zohary, 1952 ; Karimi *et al*., 2009; Karimi and Kafkas, 2011). An explanation can be found in some genetic studies performed using molecular techniques. The AFLP and SAMPL marker techniques and RAPD analysis led to postulate that *P. eurycarpa* may be an hybrid between *P. vera* and *P. khinjuk*, and that *P. atlantica*, and *P. atlantica* subsp. *cabulica* are descendents of *P. eurycarpa* (Kafkas and Perl-Treves, 2001; 2002; Al-Saguir *et al*., 2006; Karimi *et al*., 2009; Karimi and Kafkas, 2011).

3.3.2 *Correlation between climatic factors and morphological characters*

The leaf shape and structure are defined mainly in a brief period of primary morphogenesis based on the possible role of reaction–diffusion systems and can be altered by the allometric expansion (Franks et al. 2000; Dengler and Kang 2001). The final size of a leaf depends on cell division and expansion. Any factor that can influence the number and size of leaf cells may affect the dimensions and size of the leaf (Tsukaya, 2003). Phenotypic plasticity also occurred to produce a range of leaf traits that are environmentally affected (Kessler and Sinha 2004; Barkoula *et al*., 2007). In many plants, morphological characters are developed as a response to climatic factors (Meinzer *et al*. 1985; Körner *et al*., 1989; Stenström *et al*. 2002). The leaf size tends to decline with decreasing mean annual rainfall (Beard 1945; Cowling and Campbell 1980; Givnish 1984; Stone and Bacon 1995; Wolfe 1995).

In the current study, the leaf measurements are mostly correlated with climatic factors. The leaf length and width and the terminal leaflet length are strongly correlated with the precipitation and the altitude. The largest leaves and leaflets are observed mainly in the temperate sites, while in the dried sites the leaves and the leaflets are mostly narrows. The leaves become narrower and thought to be an adaptation to xeric environments (the narrow leaves could reduce the transpiration) (Xu *et al*., 2008). Besides, the leaflet thickness is negatively correlated to the mean temperature and the minimum temperature, in agreement with Ait said *et al.* (2011) who showed (in the same species) correlations between the morphological, the anatomical measurements and aridity. On other taxa, it has been demonstrated that leaf thickness increases through an increase in cell size when development takes place at low temperature (Wilson and Cooper, 1969; Ludlow 1983; Yang *et al.* 2011). Moreover, the climatic factors can have a synergetic interaction and many environmental factors (biotic,eg. competition and abiotic, eg. grazing,) can interact (Douglas 1981).

The factors that affect leaf colour change are multiple and somewhat interrelated. The larger external causes are temperature, light levels, wind, and precipitation. Internal factors include photosynthetic rate, respiration rate, and protein levels. The increase in light intensity leads, through two ways, to the intensity of colour leaves. At the chloroplast level, the increase in light intensity causes an increase in the overall content of chlorophyll per unit leaf area by increasing the foliar volume and the number of chloroplasts. While in the leaf level, the increase in light intensity induce a limb thickening which leads to increase in chloroplast number by increasing the cellular layers in the palissadic parenchyma. The leaf colour intensity increases also at altitude

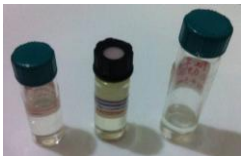
gradient due to the increase in the content of chlorophyll (Chl a & Chl b) and of flavonoids. Flavonoids also facilitate absorption of light (Lichtenthaler *et al*., 2007; Rajsnerová *et al*., 2015). Furthermore, flavonoids in the leaf epidermis absorb ultraviolet light and protect chlorophyll from photo-oxidation. It follows that high elevation plants receive a greater amount of UV radiation and have more flavonoids (Rajsnerová *et al*., 2015). This is why alpine flowers have such intense colour. This may explains the dark green colour observed in the sites AF, OM, and BT characterized by a high altitude. The same case was stated by Belhadj *et al*. (2008). The leaves exposed to light for a long time produce more polyphenols (Meyer *et al*., 2006). These compounds emit a blue-green fluorescence (BGF) between 400 and 600 nm as a response to environmental factors (Cerovic *et al*., 1999). However, Murchie and Horton (1997) showed that these mechanisms are not universal mainly in the case of leaves with a phenotypic plasticity. Thus, very dark green leaves were found in the site CH at an altitude of 354 m. This site was characterized by an open stand where no concurrence occurs and the trees are largely profiting from the light intensity. Conversely, the chlorophyll pigment rate decreases at drought gradient (Fahmi *et al*., 2011) and when plants are exposed to drought stress which leads to a pigment oxidation. Leaves are so light green. This phenomena was observed on *P. mutica* (*P. atlantica* subsp. *mutica*) and *P. khinjuk* by Ranjbarfordoei *et al.* (2001).

3.4 Conclusion

In the current chapter, the morphological characterization was carried out to examine the morphological and the morphometric leaf variability. Two descriptors were used; tree descriptors and leaf descriptors showing a high level of variability. The trees are mostly robust, vigorous, with single trunk and dense foliage. However, *Pistacia atlantica* was also observed branched from base and with an intermediate vigour. The morphological and morphometric leaf characterization showed an important inter and intra-populational variability and the ANOVA analysis applied on the leaf measurement showed highly significant difference. The leaflet thickness measurement revealed variable values and the ANOVA analysis showed highly significant difference between the sites. The leaf colour was very variable ranging from the light green (the most frequent colour) to the very dark green colour. In this chapter, a significant variance at the phenotypic level was reported between individuals within the same site and between the different sites. It was demonstrated that the morphological characters were significantly correlated with climatic factors. Furthermore, the data obtained in this study was used to update the key determination of the subspecies.

CHAPTER 4
INTRASPECIFIC CHEMICAL VARIABILITY OF *Pistacia atlantica* Desf. subsp. *atlantica* ESSENTIAL OIL FROM NORTHWEST ALGERIA

The findings of this chapter were discussed at the '5th International Congress on Medicinal and Aromatic Plants'. CIPAM2014. ZARZIS, TUNISIA: 17-20 March. (El Zerey-Belaskri *et al*., 2014b).



4 INTRASPECIFIC CHEMICAL VARIABILITY OF *Pistacia atlantica* Desf. subsp. *atlantica* LEAF ESSENTIAL OIL FROM NORTHWEST ALGERIA

4.0 Background

During the last century, phytochemical studies have known a great deal of change, not only for the data they provide on the chemical compositions and for the described compounds but also in valuation prospects of these compounds. The evolution of this area resulted therefore a relevant development in other fields (pharmacology, biochemistry, paleobotany, plant physiology, ethnography and mainly systematic). Phytochemistry has made considerable progress, most likely due to the rapidly advancing of chromatography techniques.

In plants, if basic metabolisms comprise all pathways necessary for the survival of the cells, secondary metabolisms are products that occur usually only in special, differentiated cells and are not necessary for the cells themselves but may be useful for the plant as a whole (Kössel, 1891). Plant secondary compounds have multiple biotic and abiotic functions. They are well known to provide UV protection (Close and McArthur, 2002) or carbon/nutrient balance adjustment in response to sunlight (Shure and Wilson, 1993), and to act as a chemical defense against herbivores and pathogens (Levin, 1976; Bryant *et al*., 1991). They may modulate interactions with competing plants (Rice, 1979; Ehlers and Thompson, 2004) and pollinators (Beker *et al*., 1989; Ayasse *et al*., 2000). Although the functions of many secondary metabolites are already well-known; for the greatest part of them, the value to the plant is still unknown (Evans, 2002). Secondary metabolites are extremely diverse and produced sometimes as a characteristic mix that can be used in certain case as taxonomic characters in classifying plants.

Secondary metabolites including 'the Essential oils' (natural aromatic and volatile compounds) are found in many parts of plants. Essential oil, also defined as essence, volatile oil, etheric oil or aetheroleum, is a complex mixture of volatile constituents biosynthesised by living organisms (Hüsnü *et al*., 2007). If higher plants are the best-known and most important source of essential oils, some mosses, liverwrots, seaweeds and some terrestrial and marine animals (sponges), insects, fungi, and microorganismes are also known to biosynthesise volatile compounds (Katayama, 1955; 1962; Joshi and Gowda, 1975). According to the International Standard Organization on Essential Oils, ISO 9235 (1997) of the ISO/TC, the concept of essential oil is restricted to the products obtained exclusively by distillation of plant material, with or without water steam, or by mechanical processes applied to the epicarp of fruits of the genus *Citrus* L.

Essential oil is defined by the "*Association Française de Normalisation*" (AFNOR, 1998) and the European Pharmacopoeia (Eur. Ph., 2008) as "an essential oil is clearly defined as a manufactured product from pure, identified raw materials of plant origin, obtained by steam distillation, mechanical processes (e.g., EO from *Citrus*), or by 'dry' distillation for some woods". Essential oils may comprise volatile compounds of terpenoid or non-terpenoid origin. All of them are hydrocarbons and their oxygenated derivatives. Nitrogen or sulphur derivatives may exist in the form of alcohols, acids, esters, epoxides, aldehydes, ketones, amines, sulphides, *etc* (Connolly and Hill, 1991; 2005; Dewick, 1999; Degenhardt *et al*., 2009). As in most of living beings, the secretion of different substances occurs in specialized cells. Essential oil is secreted in many types of structures (secretory structures) (Svoboda and Svoboda, 2000; Ascensão, 2007). In Anacardiaceae, substance secretion such as gum, resin and oil occurs in the ducts (Muller 1866-1867; Sieck, 1895; Engler, 1896; Tschirch, 1900). In fact, Secretory ducts (Fig. 4.1) are characteristic of this family (Joel *et al*., 1978; Carmello-Guerreiro and Sartori Paoli, 2002). They are located both in the primary and secondary phloem (Metcalfe and Chalk, 1950). In Anacardiaceae, beside the single apotropous ovule per locule, the vertical resin ducts in the phloem is the second character distinguishing the family from the Burseraceae (Metcalfe and Chalk, 1950; Joel *et al*., 1978. Carmello-Guerreiro and Sartori-Paoli, 2002).

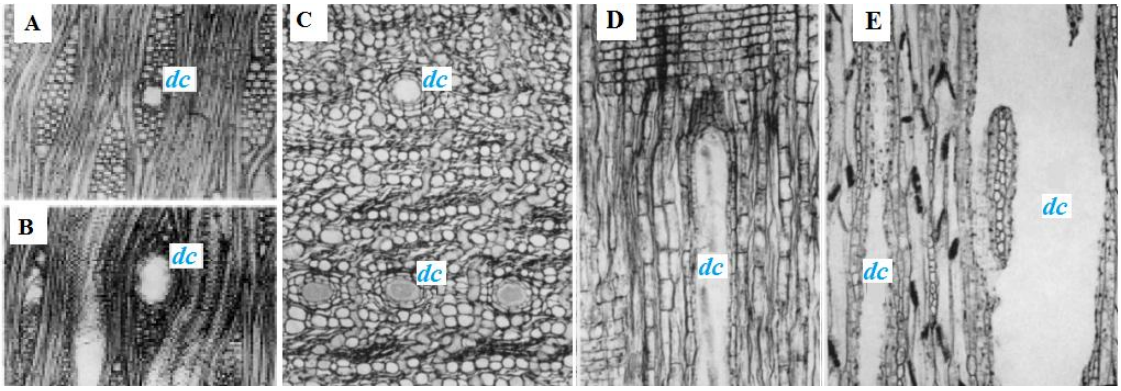


Fig. 4.1 Photomicrographs of secretory ducts (***dc***) in secondary phloem in some Anacardiaceae: in *Pistacia* sp. (A, B) (El-Oqlah, 1996), in *Rhus glabra* (C, D, E) (Fahna and Evert, 1974)

Chemical markers, such as essential oil compounds are being widely used in taxonomy as well as for biodiversity studies (Ge *et al*., 2008). Their quail-quantitative status varies through the ontogenetic development and is under the influence of abiotic and biotic environmental factors (Adams, 1998; Demetzos *et al*., 2002; Dong *et al*., 2011; Tavares *et al*., 2014).

Purposes

The purposes of this chapter are the quantitative and qualitative characterization of *Pistacia atlantica* subsp. *atlantica* essential oil in different populations; the investigation of inter-population chemical variability of *Pistacia atlantica* subsp. *atlantica* essential oil; and the analysis of the environmental factors effect on the chemical variability.

4.1 Material and Methods

4.1.1 *Plant Material*

Healthy leaves were collected from male and female trees in March 2013 and air-dried in the shade. samples were coded with 'capital letters' which correspond to the names of the different sampling sites BTM, CH; OR; AF1; AF2; OM; TA; BB; SBA; MBB; SF1; SF2; BT; GR; BH; 3R. The codes were followed by 'm' or 'f' when the sample came from male or female tree, respectively.

4.1.2 *Essential oil extraction and analysis*

Essential oils were isolated by water distillation (Fig. 4.2, A) for 3h, in two continuous cycles (from 200gr of dried materiel/cycle), using a Clevenger-type apparatus according to the procedure described in the European pharmacopoeia (Council of Europe, 1997). Essential oil extraction was done in the laboratory '*Biodiversité Végétale: Conservation et Valorisation, Faculté des Sciences de la Nature et de la Vie, Université de Sidi Bel Abbes, Algérie'*. Oils were kept in dark at 4°C until analysis.

4.1.3 *Gas chromatography (GC)*

The following analysis was performed at the Laboratory of Pharmacognosy, Faculty of Pharmacy CEF, University of Coimbra, Portugal.

Analytical GC was carried out using a Hewlett Packard 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph with HP GC ChemStation Rev. A.05.04 data handling system, equipped with a single injector and two flame ionization detectors (FID). Agraphpak divider (Agilent Technologies, Part Number 5021-7148) was used for simultaneous sampling in two Supelco (Supelco Inc., Bellefonte, PA, USA) fused silica capillary columns with differentstationary phases: SPB-1 (polydimethylsiloxane 30 m × 0.20 mm, film thickness 0.20 _m), and SupelcoWax 10 (polyethyleneglycol 30 m×0.20 mm, film thickness 0.20 _m). Oven temperature program: 70–220 ◦C (3 ◦C/min), 220 ◦C (15 min); injector temperature: 250 ◦C;

carrier gas: helium, adjusted to a linear velocity of 30 cm/s; splitting ratio 1:40; detectors temperature: 250 ◦C.

4.1.4 *Gas chromatography–mass spectrometry (GC–MS)*

Analyses were carried out using a Hewlett Packard 6890 gas chromatograph fitted with a HP1 fused silica column (polydimethylsiloxane 30 m×0.25 mm, film thickness 0.25 _m), interfaced with an Hewlett Packard mass selective detector 5973 (Agilent Technologies, Palo Alto, CA, USA) operated by HP Enhanced ChemStation software, version A.03.00. GC parameters as above; interface temperature: 250 ◦C; MS source temperature: 230 ◦C; MS quadrupole temperature: 150 ◦C; ionization energy: 70 eV; ionization current: 60 _A; scan range: 35–350 u; scans/s: 4.51.

4.1.5 *Qualitative and quantitative analyses*

The identity of the compounds was achieved from their retention indices on SPB-1 and SupelcoWax 10 columns and from their mass spectra. Retention indices, calculated by linear interpolation relative to retention times of C8–C22 n-alkanes, were compared with those of authentic samples included in Pharmacognosy laboratory database (University of Coimbra). Acquired mass spectra were compared with corresponding data of components of reference oils and commercial available standards from a home-made library or from literature data (Joulain and Konig, 1998; Adams, 2004). Relative amount of individual components was calculated based on GC peak areas without FID response factor correction. The relative percentage of components which co-elute on apolar column was calculated on SupelcoWax 10 column.

4.1.6 *Statistical analysis*

To analyze the chemical variability of *P. atlantica* essential oils and taking into account the compounds which registered a percentage equal or higher than 2%, 775 data (25 constituents x 31 individual) were submitted to multivariate statistical analysis using Statistica (Copyright© Stat Soft.Inc. 1984-2008). Among the total chemical composition, twenty-one main constituents were selected and submitted to the principal-components analysis (PCA). Sixteen constituents over 1.0% were used as variables for analysis. Cases were classified by a hierarchical clustering using Ward linkage with square Euclidean distance measure. Factor analyses using the principal components extraction method (PCA) was used to achieve the most relevant variables in the total variance.

4.2 Results

Pistacia atlantica leaves gave a clear essential oil with a light yellow colour (Fig. 4.2, B). The essential oil yield obtained in this study varies from 0.11 to 0.42%. The qualitative and quantitative analyses results are shown in Table 4.1, totaling 71 compounds, accounting for 76.9 to 98.6 % of the volatile oil constituents. Thirteen constituents (followed by * in the table 4.1) have never been reported in the composition of *P. atlantica* essential oils (Barrero *et al*., 2005; Tzakou 2007; Mecherara-Idjeri *et al*., 2008; Gourine *et al*., 2010a, b; Ait Said *et al*., 2011a).

The ratio monoterpenes/sesquiterpenes was different among the samples. The monoterpenes hydrocarbons (Fig. 4.3) were the main group of constituents (88.2-39.4%) in 28/31 of the analyzed samples. The sesquiterpenes hydrocarbons (34.4-34.2%) were predominant in two samples, while the oxygen containing sesquiterpenes (35.4%) and the monoterpenes hydrocarbons (35.9%) were the main groups in one sample.

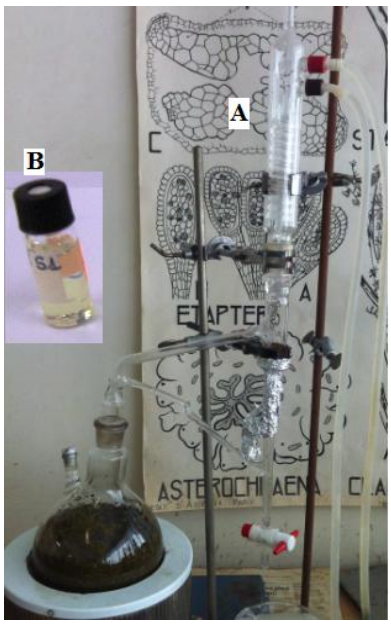


Fig. 4.2 Essential oil extraction: (A) by hydrodistillation;
(B) a light yellow essential oil

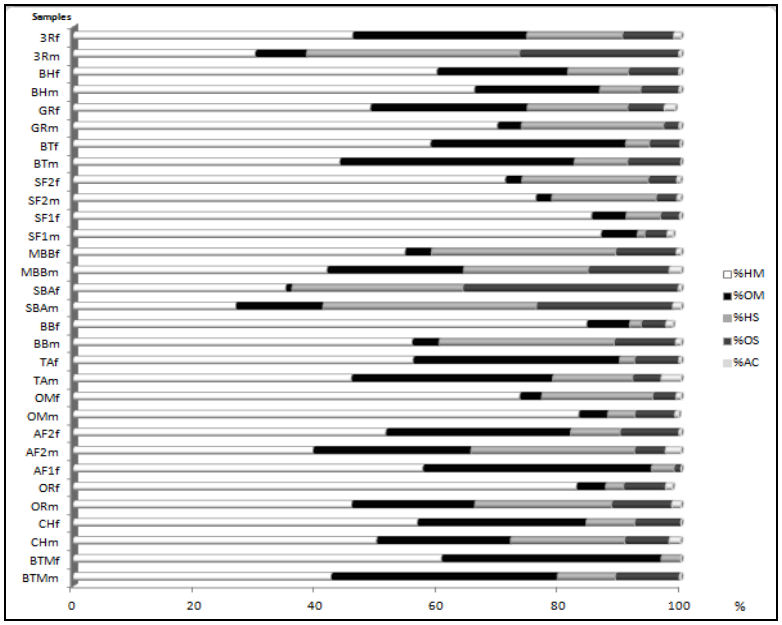


Fig. 4.3 Variability distribution of Hydrocarbon monoterpenes (HM), Oxygen containing monoterpenes (OM), Hydrocarbon sesquiterpenes (HS), Oxygen containing sesquiterpenes (OS) and Aldehyde compounds (AC) in *Pistacia atlantica* subsp. *atlantica* essential oils

β-pinene, sabinene, α-terpinene, δ-cadinene, spathulenol, myrcene, α-cadinol. The multivariate analysis data (Fig. 4.4) allowed us to establish two main types of essential oils: terpinen-4-ol (cluster I) and α-pinene (cluster II). In the last type, two subgroups could be differentiated: α-pinene/camphene (cluster II.1) and α-pinene/germacrene D (cluster II.2). The essential oils of cluster I (65.5% of the samples) are characterized by a significant content of terpinen-4-ol (mean 22.6%, S.D. = 6.53). While the chemical composition of samples of cluster II (35.5% of the samples) is characterized by a high content of α-pinene (47.5%, S.D. = 8.48), an appreciable percentages of camphene in the cluster II.1 (mean 9.9% S.D. =2.7) and germacrene D in the cluster II.2 (mean 15.5% S.D. =2.38). The principal-components analysis (PCA) allowed to put the samples into three principal groups according to the four major compounds (Fig. 4.5). The first group includes fifteen samples (15/31) defined by Terpinen-4-ol type. The second group includes thirteen samples (13/31) being α-pinene the main compound. Three samples (3/31) are gathered in the third group on the base of their major compounds germacrene D and *E*-caryophyllene.

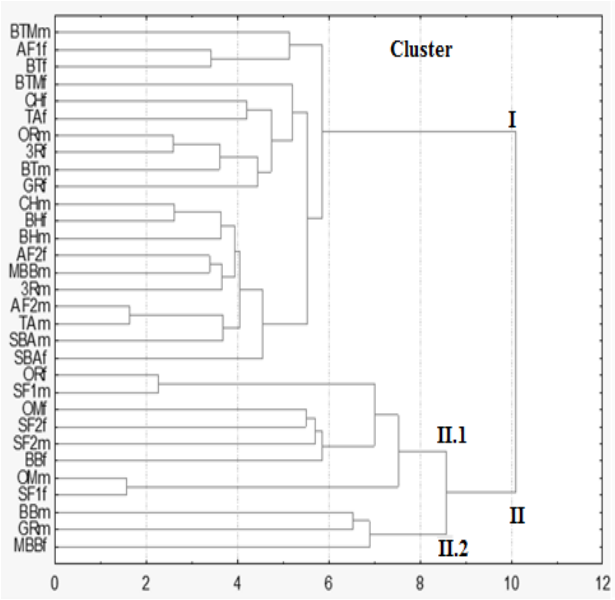


Fig. 4.4 Two dimensional dendrogram obtained in the hierarchical cluster analysis of the essential oils of individual plants of *Pistacia atlantica* subsp. *atlantica*. Horizontal: similarity levels between samples. Vertical: samples analysed

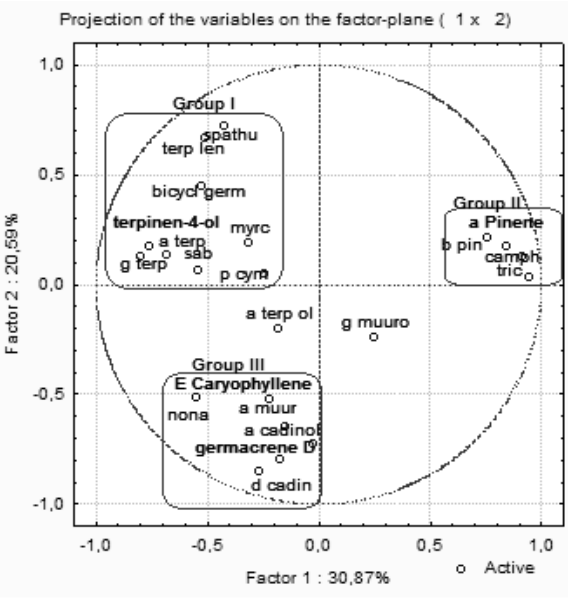


Fig. 4.5 Two dimensional PCA of the main constituents of the essential oil chemical compositions of individual plants of *Pistacia atlantica* subsp. *atlantica*

The PCA, applied separately to the female and male data (Fig. 4.6), shows an important chemical variability. The positive side of the axis 1 in Fig. 4.6 (a) and the negative side of the axis 1 in Fig. 4.6 (b) retain the 'group I' which is characterized by terpinen-4-ol as a major compound. In the male essential oil composition (Fig. 4.6 (b)), spathulenol, bicyclogermacrene and terpinolene were correlated to terpinen-4-ol, type. However, these compounds have formed an independent group 'Group III' in the female composition (Fig. 4.6 (a)) characterized by a correlation with γ-terpinene. In the negative side of the axis 1 in the female PCA (Figure 3 (a)) and the positive one of the male PCA (Fig. 4.6 (b)), a second group (GroupII) is defined on the basis of the major compound 'α-pinene'. A particular group (Group III in Fig. 4.6 (b)) appears in the male PCA with two main compounds 'germacrene D and *E*-caryophyllene'.

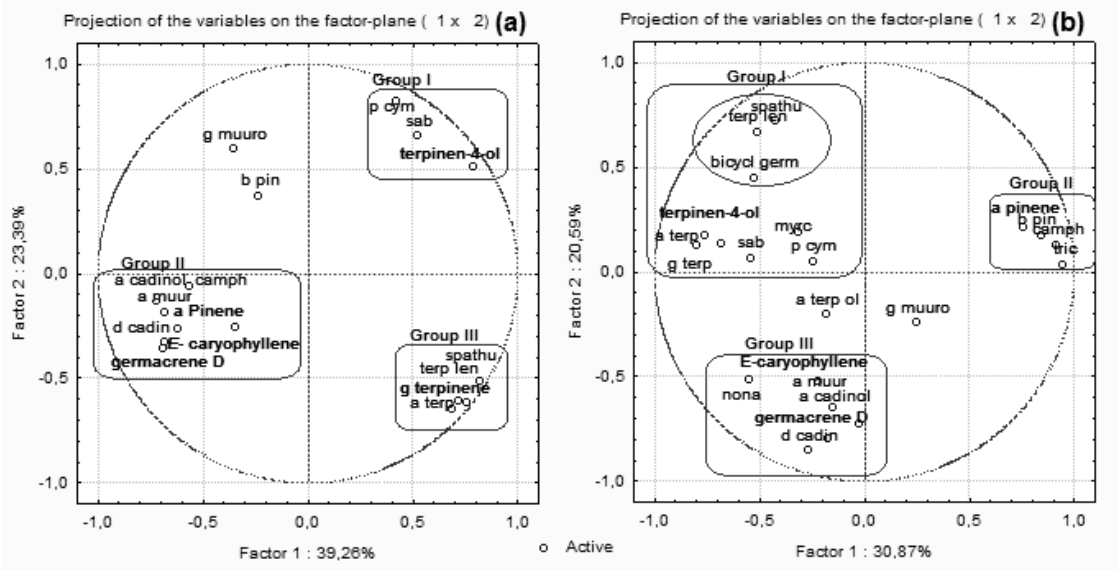


Fig. 4.6 Two dimensional PCA of the main constituents of the essential oil chemical compositions of individual plants of *Pistacia atlantica* subsp. *atlantica;* (a): Female composition, (b): male composition

4.3 Discussion

The chemical composition of the *Pistacia atlantica* essential oil was investigated previously in many studies which give continuously news data (Barrero *et al*., 2005; Tzakou 2007; Mecherara-Idjeri *et al*., 2008; Gourine *et al*., 2010a, b; Ait Said *et al*., 2011a).

Essential oil yield obtained in this study is clearly higher than that obtained from the southern Atlas pistachio populations in Algeria (Mecherara-Idjeri *et al*., 2008; Gourine *et al*., 2010b; Ait Said *et al.*, 2011a). We note that this difference could be due to two factors, the climatic conditions and the sampling period. In fact, our sampling area is under sub-humid to semi-arid

bioclimate and the leaves were harvested in March (spring season). All the works previously cited are done on populations under arid to saharian bioclimate and the leaves have been collected between june to october (Summer/Autumn seasons).

Data obtained from PCA distinguished two groups which corresponds to the samples representing the chemotype 'Terpinen-4-ol' known for the Atlas pistachio in Morroco (Barrero *et al.*, 2005), in Greece (Tzakou, 2007), and recently in Algeria (Gourine *et al*., 2009) and the samples representing the chemotype 'α-pinene' known for this species (Gourine *et al*., 2009). The latter author describes the type α-pinene/camphene however; the present study reveals two types in the chemotype (α-pinene'), α-pinene/camphene and a new type α-pinene/germacrene D. Germacrene D and *E*-caryophyllene, recorded as major compounds in three samples and recorded among the main constituents in other samples, have never been identified before as major compounds in the essential oils of *Pistacia atlantica*. This group may characterize the northern populations of *P. atlantica* in Algeria.

The current investigation showed an inter/intra-populational chemical variability in *Pistacia atlantica* subsp. *atlantica* essential oil. In some sites (such as OR, SBA, MBB, GR, 3R), the male and the female compositions belong to different chemical types. In this case, the individual sex may be targeted to explain the essential oil variability. However, for the other sites, both male and female compositions were part of the same group. In agreement with our results, Tzakou *et al*., (2007) reported that the chemical composition of *P. atlantica* essential oil, from male and female individual, may present the same major compounds as it can vary depending on the sex of the individuals. The second effect we can look for is the climate. Many studies mentioned that essential oil production and that of secondary metabolites in general is extremely dependent on weather conditions (Reeve, 2005; Selmar *et al*., 2013). Turtola *et al*., 1995 showed that under induced stress conditions of drought the total amount of terpenes and resin acids increased. However, it was not possible in the current study to show the effect of the climatic conditions; mainly when individuals under similar environmental conditions recorded variable chemical compositions (eg. OR samples), and conversely, individuals in different conditions belonged to the same chemical type (eg. ORf/SF1m) (Fig. 4.7).

In this study, two new major compounds (Germacrene D, *E*-caryophyllene) were recorded. Germacrene D was observed in a high content in many samples and as major constituent in SBAm and 3Rm. Germacrene D is found with a high account in Atlas pistachio population under a high

aridity in the southwest Algeria (Ait Said *et al*., 2011a). The authors explain the high concentration by water stress. It has been reported that the content of germacrene D from *Pistacia lentiscus* increased four times during the summer season compared to spring Gardeli *et al.* (2008).

Nevertheless, it is not the case in this study, the individuals of this group occur closely to rivers and don't seem be under water stress. As for our opinion, we suppose that it may be due to two factors. The first is about the age, since these individuals are among the oldest ones. A high percentage of germacrene D was recorded in the chemical composition of the Atlas pistachio of the botanical garden of the National Institute of Agriculture (Algiers-Algeria) (Mecherara-Idjeri *et al*., 2008), which is an old specimen. The second factor is related with insect attack. Plants of the genus *Pistacia* serve as obligate hosts for a group of specialized gall forming aphids (*Homoptera*: *Fordinae* and *Aphididae*) (Inbar, 2008). During our investigation, two month after collecting the leaves, we have noticed that these populations are the most attacked by the insects. Germacrene is the bitter sesquiterpene olefins produced by a number of plants representing compounds that possess antifeedant, antimicrobial and insecticidal properties (Govindarajan, 2010; Sosa *et al*., 2012). Germacrene-D was identified in the oleoresin of *P. atlantica* harvested in the same study area by Benhassaini *et al.* (2008), while it was not noted in the oleoresin of the other subspecies. This compound is very common in different species of the genera *Pistacia* (Couladis *et al*., 2003; Roitman *et al*., 2011; Bozorgi *et al*., 2013) and *Schinus* (Anacardiaceae), and in *Commiphora* and *Bursera* of the Burseraceae family which is closely related to the Anacardiaceae (Gadek et al., 1996). Noge and Beccera (2009) suggest that producing germacrene D might be an ancient trait in the genus *Bursera*. This theory might be projected on the Anacardiaceae.

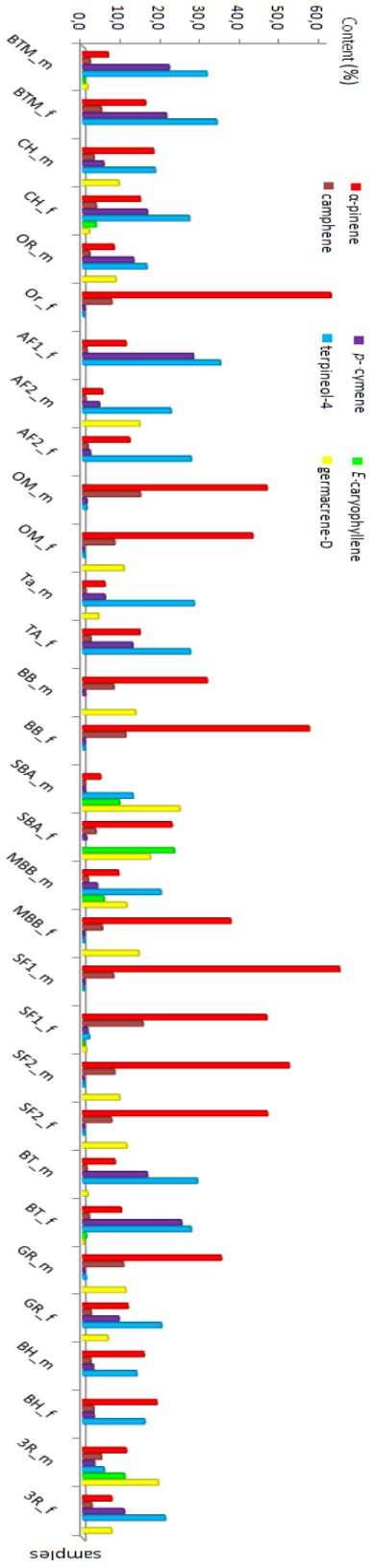


Fig.4.7 Main compounds variation in leaf essential oils of 33 *Pistacia atlantica* subsp. *atlantica* samples

This component could be identified with high content in other samples of the natural populations. However, *E*-caryophyllene has never been identified in a high percentage in *P. atlantica* essential oil, it is an important constituent in the essential oils of some species of the Anacardiaceae family, such as *P. lentiscus* (Ait Said *et al*., 2011b), *Anacardium humile* and *A. occidentale* (Montanari *et al*., 2012) and *P. terebinthus*. *E*-caryophyllene is a biociclic-sesquiterpene, with strong woody and spicy odor, widely used in cosmetology and perfumery for its aroma quality (Skold *et al.*, 2006). Caryophyllene is known for its anti-inflammatory effect (Ghelardini *et al.*, 2001; Fernandes *et al*., 2007; Michielin *et al.*, 2009) and antiallergic effect (Michielin *et al.*, 2009). It has an analgesic activity and cytoprotectif gastric effect (Ghelardini *et al*., 2001). It is very used in medical and pharmacological fields. Especially in the locations where this compound has a high account in the essential oils, the leaves of the autumn season are often used by the local population as an efficient treatment for stomach and gastric diseases (according to our own ethnobotanical survey, data not shown). Moreover, this compound is approved by the *U.S. Food and Drug Administration* as food additive.

So, more investigations in the populations of this group (third type) may bring out a new chemotype for this species.

4.4 Conclusion

In this chapter, leaf essential oil obtained from male and female from sixteen sites was characterized showing a significant chemical variability. Variance was observed within site and between sites. The essential oil yield obtained in this study varies from 0.11 % to 0.42%. The essential oil was analyzed by CG followed by CG/MS which reveals a total of 71 compounds with 13 constituents never reported in the composition of *Pistacia atlantica* essential oils. The multivariate analysis data (hierarchical cluster analysis) allowed us to establish two main types of essential oils: terpinen-4-ol and α-pinene. The last type, two subgroups could be differentiated: α-pinene/camphene and α-pinene/germacrene D. The principal-components analysis (PCA) allowed to distinguish three groups according to the four major compounds (Terpinen-4-ol, α-pinene, germacrene D and *E*-caryophyllene). The two last compounds were never cited as major compound for this species. These compounds may characterize the northern populations of *P. atlantica* in Algeria.

CHAPTER 5
QUANTITATIVE CHARACTERIZATION OF CELL WALL POLYSACCHARIDES FROM *Pistacia atlantica* Desf. subsp. *atlantica* LEAVES THROUGH CELLULOSE AND HEMICELLULOSES DOSAGE

Part of this chapter is published as 'El Zerey-Belaskri A., Benhassaini H., Naimi W. and Rahoui S. 2013. Cellulosic and hemicellulosic fractions dosage of *Pistacia atlantica* Desf. subsp. *atlantica* leaves in western Algeria, *Natural. Product. Research*.: Formerly Nat. Prod. Let. **27**:19, 1757-1763, doi:10.1080/14786419.2012.755679'.

5 QUANTITATIVE CHARACTERIZATION OF CELL WALL POLYSACCHARIDES FROM *Pistacia atlantica* Desf. subsp. *atlantica* LEAVES THROUGH CELLULOSE AND HEMICELLULOSES DOSAGE

5.0 Background

Cell walls are essential to many fundamental processes in plants, such as growth and resistances plant. Most of plant cells produce a primary wall which is much thinner than cytoplasmic membrane. Certain cells deposit additional layers inside the primary wall to form a secondary wall. Polysaccharides constitute a major proportion of plant cell wall (McNeil *et al*., 1984; Carpita and Gibeaut, 1993), depending, of course, upon the species and the tissue under consideration. Cell walls define mainly the structure of plants. They play role in plant defense against pathogens. Although, all cell walls consist of celluloses, hemicelluloses, and pectins polysaccharides (McNeil *et al*., 1984). The proportions of each vary from species to species and with cell type. The variability can be observed also between populations of the same species. Cellulose occurs as crystalline, fibrillar aggregate of b-1,4-linked glucan chains (Frey-Wyssling, 1969; Carpita and Gibeaut, 1993). The term hemicellulose refers to a group of homo- and heteropolymers consisting largely of anhydro-β-(1→4)-D-xylopyranose, mannopyranose, glucopyranose, and galactopyranose main chains with a number of substituents (Timell, 1965). Hemicelluloses are generally found in association with cellulose in the secondary walls of plants, but they are also present in the primary walls.

Those polysaccharides have a great practical significance including important role in the material industry and in human and animal health as dietary fiber (Belhamri, 2005; Zeitoun, 2011). In addition, various lignocellulosic crops, non-recoverable for human food, are tested for the production of renewable energy vectors. The lignocellulosic biomass has been recognized as a major world renewable energy source to supplement declining fossil fuel resources (Ozçimen and Karaosmanoglu, 2004). It is used as feedstock for second generation bioethanol production. The energy recovery potential efficiency of the lignocellulosic crops depends on their content in cellulose and hemicelluloses (Carpita *et al*., 2000; Houghton *et al*., 2006; Godin *et al*., 2010). In Turkey, Pistachio (*P. vera*) shell is being considered as a very important candidate for a source of fuels and chemical feedstock (Demiral *et al*., 2009). Also in China, *Pistacia chinensis* seed oil is proposed as a promising non-edible feedstock for biodiesel production (Qin *et al*., 2012).

Pistacia atlantica is a deciduous tree. Deciduous trees are plants that drop their leaves for a part of every year, usually during periods of dryness or cold weather. Their branches remain free of foliage until conditions improve. For the tree, this means that it can save energy by not working to keep the leaves green and healthy. The researcher believes that these deciduous leaves should be valorized before leaf fall.

Purposes

Despite the significant biomass potential of *Pistacia atlantica* in the plain and steppe areas, the valuation of areal biomass of *P. atlantica* through the cell wall polysaccharides has not been studied until our work (El Zerey-Belaskri *et al*., 2013). The aim of this study is to valorize *Pistacia atlantica* leaves by the dosage of cell wall cellulosic and hemicellulosic fractions.

5.1 Material and methods

5.1.1 *Plant material*

Leaves were harvested in the period of October to December (2010 to 2013) from natural populations in the sites of BTM, CH, OR, AF, OM, TA, BB, SBA, MBB1, MBB2, SF1, SF2, BT, GR, BH, 3R. The leaves are dried at 60°C for 48 hours then ground into powder using a cutting mill.

5.1.2 *Determination of moisture content*

The moisture content of the sample material is calculated from the leave initial masse by the followed formula:

$$\% \text{ moisture} = 100 \text{ x } (\text{initial M} - \text{final M} / \text{initial M})$$

5.1.3 *Isolation of cell wall residue*

It is the first step in cell wall polysaccharides isolation. The cell wall residue was obtained according to Timell (1965) modified by Harche *et al*. (1991). So, 50 g of the powder were extracted with 100 ml methanol-chloroform (v/v), in the hood under stirring for 14 h. this step should be repeated toice in order to eliminate lipids, tannins and other cytoplasmic component. After filtration using a bolthing cloth, the wall residue was extracted with 100 ml ethanol under stirring for 2h in order to remove all traces of chloroform. After filtration, the residue was rinsed in distilled water for four times, followed by one rinse with acetone. The residue was dried in the oven at 60°C for 48h in order to determine wall residue yield.

5.1.4 *Isolation of cellulose and hemicelluloses cell wall*

The cellulose and the hemicelluloses cell wall were isolated according to Chanda *et al.* (1950). This protocol is very simple and it does not require any special equipment. So, 5 g of the wall residue were extracted with 100 ml NaOH (4%) in the hood, under stirring for 14h. After filtration using bolting cloth, the filtrate (Filtrate 1) is preserved for later use. The obtained residue was extracted once again according to the same procedure. After filtration, the filtrate (Filtrate 2) is preserved for later use. The residue obtained was rinsed in distilled water for four times, followed by one rinse with acetone. The residue is dried in the oven at 60°C for one night, weighted to determine the cellulose content expressed also in percentage. The two filtrates (Filtrate 1+ Filtrate 2) previously obtained are neutralized by a pure acetic acid, precipitated in ethanol (1/3v) for one night, then centrifuged for 30 mn at 3600 r.p.m. The sediment is rinsed in distilled water for four times, followed by one rinse with acetone. The obtained residue is dried in the oven at 60°C for one night then weighted to determine the hemicelluloses content expressed also in percentage.

5.1.5 *Statistical analysis*

The data concerning the moisture content and the wall residue yield are submitted separately to one-way analysis of variance (ANOVA1, (α=5%)). The data concerning the cellulosic and the hemicellulosic fractions are submitted to two-way analysis of variance (ANOVA2, (α=5%)). The correlation between the leaf morphological characters and the data obtained in the present work is checked by the correlation coefficient (*co*).

5.2 Results

5.2.1 *Determination of moisture content and wall residue yield*

The moisture content is determined vs. the initial weight of fresh material. Looking at the overall percentage of moisture composition (Table 5.1), it ranged between 36.54 % and 70.21 % and was highest in the samples of MBB2 and MBB 1 followed by SBA and AF. The samples of BT and BH recorded the lowest moisture content. The data concerning the moisture content submitted to one-way analysis of variance indicate a significant difference between the different

Table 5.1 Means of moisture content and wall residue yield in mature leaves (expressed in percentage ± sd)

Sites	Mean of moisture content (%±sd)	Mean of wall residue Yield (%±sd)
BTM	43.8±0.12	54.85±0.64
CH	40.96±0.96	42.58±3.84
OR	45.16±2.99	45.97±0.53
AF	54.9±1.09	53.14±1.97
OM	47.83±0.47	45.86±0.64
TA	44.54±0.54	45.91±5.96
BB	40.69±1.5	69.5±0.03
SBA	57.7±0.79	49.65±1.69
MBB1	68.71±0.95	59.78±0.83
MBB2	70.21±1.05	63.7±0.5
SF1	44.54±1.42	39.06±0.76
SF2	42±0.98	48.52±0.09
BT	36.54±0.54	46.4±0.32
GR	43.6±0.4	36.2±0.71
BH	36.82±0.18	43.8±3.85
3R	54.27±0.73	47.51±5.93

sites (*P = 0.02, P <0.05*). The wall residue yield is determined vs. the initial weight of plant powder. The wall residue yield is variable and ranges from 36.2 % (in GR) to 69.5% (in BB) (Table 5.1).Others highest yields are recorded in MBB2 and MBB1 and in BTM. The data concerning the wall residue yield submitted to one-way analysis of variance indicate a significant difference between the different sites (*P = 0.03, P<0.05*).

5.2.2 *Determination of cellulose and hemicelluloses cell wall yields*

The cellulose and hemicelluloses cell wall yields (Fig. 5.1) are determined vs. the initial weight of wall residue.

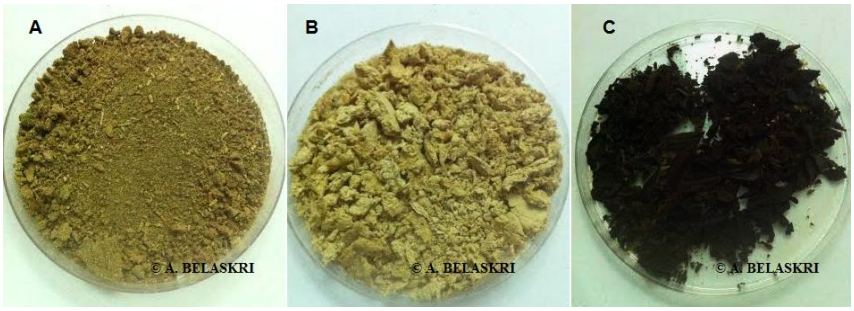


Fig. 5.1 Extracted cell wall polysaccharides from *Pistacia atlantica* subsp. *atlantica* leaves: A : Wall residue, B: Wall Cellulose, C: Wall hemicelluloses

The dosage of the two fractions shows that the cellulosic fraction is more important than the hemicellulosic fraction (Fig. 5.2). The cellulose cell wall yield is variable and ranges from 21.25% to 51% (Fig. 5.3). The highest yields are noted in SBA, BT and TA; while the lowest yield is recorded in SF1. The hemicelluloses cell wall yield varies from 7% to 27.6% in the mature leaves (Fig 5.4). The highest yields are noted in OR, BTM and SBA; while the lowest yield is recorded in MBB1 and TA.

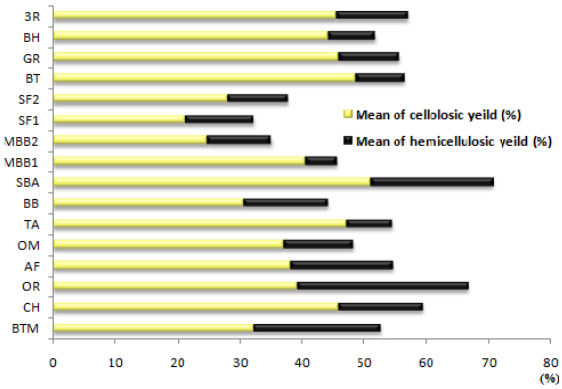


Fig. 5.2 Cellulosic fraction dominance compared to hemicellulosic fraction in *Pistacia atlantica* subsp. *atlantica* mature leaves

The data concerning the two fractions submitted to two-way analysis of variance indicate a very high significant difference between the cellulosic fraction and the hemicellulosic fraction *(P=1.0951E-07),* while no difference was recorded between the different sites both for the cellulosic and the hemicellulosic yields *(P=0.45).* A negative weak correlation is recorded

between the wall residue yield and the cellulosic yield (r= -0.38), and a weak correlation is found between the wall residue yield the hemicellulosic yield (r=0.13). The correlation checked with the morphological measurement indicate a negative correlation between the cellulosic yield and both leaf length (r= -0.54) and leaf width (r= -0.54), while a weak correlation is showed between the hemicellulosic yield and both leaf length (r= 0.14) and leaf width (r= 0.15).

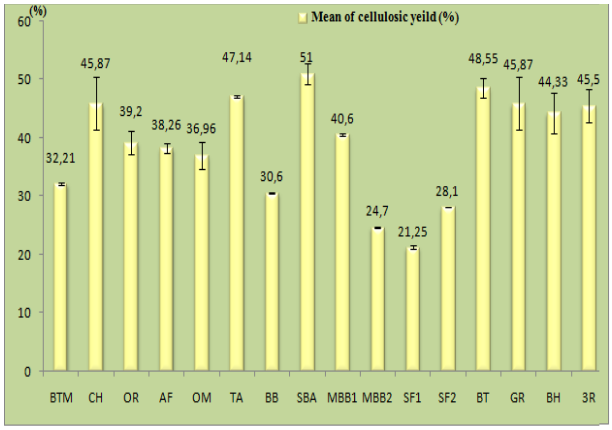


Fig. 5.3 Variation in mean of cellulosic yields in mature leaves in *Pistacia atlantica* subsp. *atlantica*

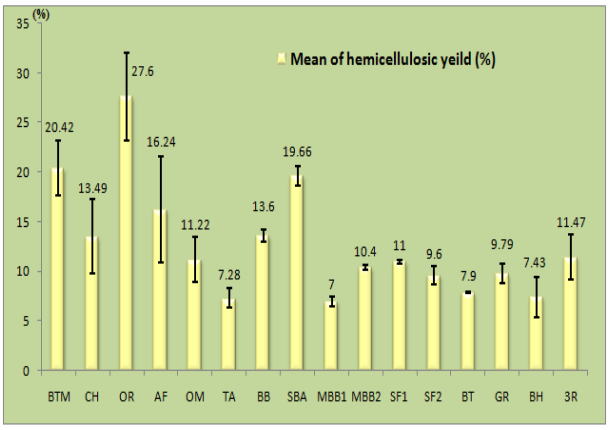


Fig. 5.4 Variation in mean of hemicellulosic yields in mature leaves in *Pistacia atlantica* subsp. *atlantica*

5.3 Discussion

The aim of this research was to highlight the cell wall cellulose and the hemicelluloses in *Pistacia atlantica* leaves. These polysaccharides are natural products with a large interest.

Cellulose is the most abundant biopolymer on Earth (Klemm *et al.*, 2005). For a long time, it is ideal for paper making but it is also used in a wide variety of areas: in the pharmaceutical industry, it is chemically treatment and transformed into a cotton wool. The chemical treatment of the cellulose provide many other products such as the cellulose nitrate used in the manufacture of explosives and the cellulose acetate used in the manufacture of the photographic films. Hemicelluloses are homo and heteropolysaccharides that the composition differs between species. They are very used as dietary fibers which are preventative products for many diseases, notably digestives diseases. In addition, hemicelluloses also form the basis of many drugs. In this research, we do not dry leaves only in order to determine the content of moisture but also in order to increase the polysaccharides yield. Indeed, previous studies showed that the chemical composition (contents of cellulose, proteins, vitamins…) is more important in the dry matter than in the fresh matter (Ndong, 2007).

The wall residue (called also the insoluble fraction) yield of the different sites is variable. The difference between the wall residue yields in the sixteen sites may be due to the variation in the climatic factors. It has been reported that under arid conditions, plants produce more insoluble polysaccharides and they store less soluble sugars (Belyea and Ricketts, 1993; Chenost and Kayouli, 1997). In other studies using the same protocols, similar wall residue yields have been obtained with other species: *Urginea pancration* (Bouzidi *et al*., 2010), and *Ammophila arenaria* (Bendimered, 2014). The plant cells have a complex structures and their compositions vary according to different factors (plant species, age, type of tissue). In the case of durum, wall residue yield is about 50% (Bertrand *et al*., 2006). In addition, Cleland (1977) note that chemical nature may be influenced and changed by phytohormones such as auxin, and consequently, the physical properties of the wall polymers to such extent that the rate of wall expansion is increased. The cellulosic and the hemicellulosic fractions vary among species. They vary within a single plant (root, stems, leaves) (Bertrand *et al*., 2006; El Bouhissi, 2011), with age, stage of growth (El Zerey-Belaskri *et al*., 2013) and with the conditions under which the plant grows (Jeffries, 1994). The cellulose yields in *Pistacia atlantica* leaves in the sites SF1 and MBB2 are comparable to those obtained by other researches (Simson and Timell, 1978a,b). However, the yields recorded in the other sites are more important.

We also note that the cellulose yields obtained in the current study are more important than the yield observed in *Stipa tenacissima* and *S. barbata* (Bessam, 2008). The later result is obtained in the same laboratory, using the same protocol. The hemicellulosic yield obtained is similar to that mentioned by several authors in *Ammophila arenaria*, *Helianthus annuus*, *Medicago sativa* (Cindy *et al*., 1987; Tissoura, 2001; Bendimered, 2014).

Generally, the cellulose is the major component of the plant residue followed by the hemicelluloses (Preston, 1986). However, young leaves contain more hemicelluloses than cellulose (Bergnans *et al*., 1996; Raynal, 1996; El Zerey-Belaskri *et al*., 2013). In fact, in the young leaves, cells are not yet producing secondary wall. In the primary wall, the hemicelluloses are very important because they give to the wall structure the elasticity which is essential to the plant growth.

5.4 Conclusion

The chemical characterization was done in the current study through the cell wall polysaccharides. The wall residue yield is variable and ranged from 36.2 % to 69.5 %. Cell wall polysaccharides

were extracted from mature leaves. The obtained yields showed that the cellulosic fraction (ranging between 21.25% and 51%) is more important than the hemicellulosic fraction (varying from 7% to 27.6%).

If *Pistacia atlantica* leaves are an important source of monoterpenes and sesquiterpenes, it appears that cell wall polysaccharides in *Pistacia atlantica* leaves open up also new opportunities to valorize the deciduous leaves in other fields.

CHAPTER 6
GENOME SIZE DETERMINATION AND CHROMOSOME ASSESSMENT IN *Pistacia atlantica* Desf. subsp. *atlantica* FROM NORTHWEST ALGERIA

6 GENOME SIZE DETERMINATION AND CHROMOSOME ASSESSMENT IN *Pistacia atlantica* Desf. subsp. *atlantica* FROM NORTHWEST ALGERIA

6.0 Background

The genetic diversity is measured in terms of amount of DNA and chromosome number, which have both biological and ecological consequences that affect the distribution and persistence of biodiversity.

The 'DNA amount' is also called the DNA C-value (Swift, 1950). Swift (1950) was the first who used the 'C value' with C for 'Constant' (see Bennett and Leitch 1995). Bennett and Smith (1976) defined the 'nuclear DNA amount' as the DNA content of the unreplicated haploid chromosome complement'. 'DNA C-value' and 'genome size' are often also used synonymously (Greilhuber *et al*., 2005). However, many ambiguities were noted in the use of all this terminology. Greilhuber *et al*. (2005) discussed the unstable usage of the terms 'genome size' and 'C-value', and proposed a new unified terminology that can describe nuclear DNA contents with ease, but without ambiguity. Greilhuber *et al*. (2005) defined the DNA content as the amount of DNA in any given cell nucleus irrespective of the state of replication, degree of endopolyploidy, etc. The 'Genome size' is, according to Greilhuber *et al*. (2005), ''covering term for the amount of DNA in the holoploid genome of an organism and also in the monoploid constituent genomes in polyploids''.

The 'Nuclear DNA amount' and the 'genome size' are important biodiversity characters with fundamental biological significance and many uses (Bennett and Leitch, 1995; Bennett *et al*., 2000).The diversity of eukaryotic genome sizes has long fascinated, but at the same time puzzled, scientists. Genome size is a highly important factor in plant biodiversity, especially in plant biology branches such as physiology, systematics, evolutionary biology, and ecology (Ohri, 1998; 2005). In prokaryotes, pulsed field gel electrophoresis and complete genome sequencing are the predominant methods of genome size determination. However, in eukaryotes, the nuclear genome size is typically measured using either densitometric measurements of Feulgen-stained nuclei (previously using specialized densitometers), now more commonly using computerized image analysis (Hardie *et al*., 2002) or flow cytometry 'FCM' (Shapiro, 2004; 2007). The last author developed originally this technology in the late 1950s for rapid counting and analysing of blood cells in clinical research and practice. It took two more decades before FCM started to be used in various fields of biological science, including experimental and field botany, with the advent of user-friendly and versatile bench-top instruments, discovery of new fluorochromes and

development of convenient protocols (Doležel *et al*., 2007; Bennett and Leitch, 2005; Doležel and Bartoš, 2005). Experimental techniques for genome size led to demonstrate high variable sizes among eukaryotic organisms. In tracheophytes, a minimum of 1C = 63.57 Mbp is present in *Genlisea margaretae* (Lentibulariaceae; Lamiales) (Greilhuber *et al*., 2006) and, for a long time, a known maximum of 1C = 124597.2 Mbp has been reported for the geophyte *Fritillaria assyriaca* (Liliaceae; Bennett and Leitch, 2005). However, two larger genomes were reported in another geophytic species, *Trillium hagae* (Melanthiaceae) with 1C = 129536.1 Mbp (Zonneveld, 2010), and *Paris japonica* (Melanthiaceae) with 1C = 148880.9 Mbp (Pellicer *et al.*, 2010).

Flow cytometry is a laser-based, biophysical technology employed in various molecular biology studies. Many applications of FCM include cell counting, cell sorting, biomarker detection and protein engineering (Leitch and Bennett, 2007; Leitch *et al*., 2009). In the current study, the FCM was used for the genome size determination of *Pistacia atlantica* subsp. *atlantica* from Northwest Algeria. Actually, flow cytometry is considered as the method of choice for genome size determination studies (Catrice *et al*., 2006; Doležel *et al*., 2007; Loureirou *et al*., 2010). Using this technique, the size of the genome is estimated by comparing the fluorescence emitted by an intercalating DNA fluorochrome of a sample together with a reference standard with known genome size. Given that a flow cytometer is available, the method provides reliable estimates of genome size in a very short period of time (10 min.) and can be considered a fast and relatively cheap alternative to other molecular tools (D'Hondt *et al*., 2011).

In spite of the systematic, cytological, and evolutionary importance of the chromosome numbers (Raven, 1975; Stuessy, 1990), the genus *Pistacia* has been scarcely studied and the most targeted species, in this way, was *P. vera*. The first chromosome investigation on the genus was done by Zohary (1952) who reported three chromosome counts (2*n*=24 in *P. lentiscus*), (2*n*=28 in *P. atlantica*), and (2*n*=30 in *P. vera*). Later, the chromosome counts were evaluated by others researchers showing sometimes different chromosome numbers in the same species (Table 6.1).

However, cytogenetic data on *Pistacia* species are still rather few. Poor protocols are used and are faced to difficulties such as the small chromosome size but also to the germination and root development constraints, a circumstance that also prevents building karyotypes (Vogt and Aparicio, 1999; Fasihi Harandi and Ghaffari, 2001; Ila *et al.*, 2003). More, for the species *P. atlantica*, most of works have been done on the subspecies occurring in Asia (Turkey and Iran), including (subsp. *cabulica*, subsp. *mutica* and subsp. *kurdica* (=*P. eurycarpa*).

CHAPTER 7
MOLECULAR CHARACTERIZATION AND EVALUATION OF INTRASPECIFIC GENETIC DIVERSITY OF *Pistacia atlantica* Desf. subsp. *atlantica* IN NORTHWEST ALGERIA, USING SSR MARKERS

7 MOLECULAR CHARACTERIZATION AND EVALUATION OF INTRASPECIFIC GENETIC DIVERSITY OF *Pistacia atlantica* Desf. subsp. *atlantica* IN NORTHWEST ALGERIA, USING SSR MARKERS

7.0 Background

Molecular markers are useful tools for analyzing genetic variation and provide efficient means to link phenotypic and genotypic variation. These markers play a key role in the studies of genetic variability and diversity and can be used to select traits that are difficult to measure using phenotypic assays (Varshney *et al*., 2005; Appleby *et al*., 2009; Kalendar *et al*., 2011).

Several genetic marker systems have been developed in the last three decades (AFLP[1], RAPD[2], ISSR[3], RFLP[4], SRAP[5] and SNP[6], SSR[7]) and have gained considerable importance in plant genetics (Poczai *et al*., 2013). Among these systems, microsatellites have been the preferred choice for various applications, such as variety identification, genetic diversity evaluation, phylogenetic analyses, genetic map construction, marker-assisted selection and comparative mapping (Beckmann *et al*., 1990; Gupta *et al*., 2000; Parida *et al*., 2009). Microsatellites (Litt and Luty, 1989) are also known as Simple Sequence Length Polymorphism (SSLP) (Tautz, 1989), Short Tandem Repeats (STRs) (Edwards *et al*., 1991) or Simple Sequence Repeats (SSRs) (Jacob *et al*., 1991), being the latter name the most commonly used.

These markers consist of motifs of 1 to 6 nucleotides repeated several times that have a characteristic mutational behaviour (Kelkar *et al*., 2010). As a consequence of their elevated mutation rates, SSRs are typically highly polymorphic: different individuals exhibit variation manifested as differences in the number of repeats. They have a frequent occurrence in all prokaryotic and eukaryotic genomes analyzed to date (Zane *et al*., 2002). The abundance of microsatellites in plants was confirmed by Delseny *et al*. (1983) and Tautz and Renz (1984). Plants are rich in AT repeats, whereas in animals AC repeat is the most common. This appears to be the general feature distinguishing plant and animal genomes (Powell *et al*., 1996). SSRs can also be found in the chloroplastic (Provan *et al*., 2001; Chung *et al*., 2006) and mitochondrial (Soranzo *et al*., 1999; Rajendrakumar *et al*., 2007) genomes. SSRs are characterized by a low degree of repetition per locus (5–100), random dispersed distribution of about 104–105 per genome (Tautz, 1993) and high degree of length polymorphism (Zane *et al*., 2002). The high

[1](Amplified Fragment Length Polymorphism), [2](Random Amplified Polymorphic DNA) [3](Inter-Simple Sequence Repeat) [4](Restriction Fragment Length Polymorphism) [5](Sequence-Related Amplified Polymorphism) [6](Single Nucleotide Polymorphisms) [7](Simple Sequence Repeat).

GENERAL CONCLUSION AND PROSPECTS

GENERAL CONCLUSION AND PROSPECTS

The current thesis focused mainly around the investigation of the variability of various characters in *Pistacia atlantica* subsp. *atlantica* and the assessment of genetic diversity of the subspecies in Algeria: a controversial topic for long time. Species with a long generation time and long lifespan delay reproductive maturity and establish variable phenotypes and genotypes within existing populations.

First of all, this work integrated the climatic characterization of the study area in which *P. atlantica* occurs in natural populations. The study area is located in Northwest Algeria and the targeted sites were chosen in longitudinal gradient from the extreme West in the region of Maghnia to the region of Mascara through the regions of Tlemcen then Sidi Bel Abbes. *P. atlantica* subsp. *atlantica* in Northwest Algeria occurs under heterogeneous climatic conditions. From 305 to 821 m of elevation, Atlas pistachio populations are under 225 mm/y to 537.36 mm/y of average annual precipitations, moderate to warmer climate (*sensu* Debrach), arid to humid climat (*sensu* De Martonne) and arid to subhumid bioclimate (*sensu* Emberger). The area is characterized by a large temperature range and a low summer drought index in many sites. The potential for a population to adapt to variation in climatic conditions will in part be governed by the average lifespan of individuals and the age at which they reach reproductive maturity. The adaptation of long-lived species to such local environmental heterogeneity is attained by the phenotypic plasticity. It is the case of *P. atlantica* in North Africa. The finding of the current study is the result of four years of field investigation aiming at characterizing the morphological variability of *P. atlatica* subsp. *atlantica* in Northwest Algeria. In this study, *P. atlantica* leaf morphology shows an important variability which is significantly correlated to the climatic conditions. New features were observed, up to 18 leaflets, up to 24.5 cm leaf length, up to 21.9 cm leaf width. The terminal leaflet may be petiolulated. In the plant, the leaves are the most exposed organs to the aerial conditions and the changes of their characters have been interpreted as adaptations to specific environments. In addition, taking into account the previous keys proposed by Al Yafi (1978) and the Al-Saghir and Porter (2012) and the new feature exposed for the first time in the leaf morphology of the subspecies, the number of leaflets and the measurement recorded on the leaf, the petiole, the terminal leaflet which surpass significantly those described in the previous literature, we proposed to update the determination key of *Pistacia atlatica* Desf. subsp. *atlantica*. At the end of this part, we believe that the morphological characterization given in this thesis, will be a helpful tool in the differentiation between *P. atlantica* subspecies where *P. atlatica* Desf.subsp. *atlantica* and the other subspecies occur together.

The essential oil of Atlas pistachio is characterized by a wide variability which may enhance the opportunities of its valorization. Two new major compounds were identified in this study, germacrene D and *E*-caryophyllene. Furthermore, thirteen constituents have never been cited in *P. atlantica* leaf essential oils. The chemical variability reported in this study points to the need to preserve the natural Atlas pistachio populations. In Algeria, pistachios branches and drupes are used as source of fuel wood. Among the multiple virtues of *P. atlantica*, its dense foliage appears promise more interests in cell wall polysaccharides fields and advantages the use of *P. atlantica* in paper industry, as raw material for biofuels production and in pharmaceutical fields. Cell wall polysaccharides in pistachio leaves have not been studied much until now. For that reason, the current study is a prospective study in order to judge to the opportunity to any potential exploitation and valuation.

The current study reported for the first time the genome size of *P. atlantica* and the chromosome count of *P. atlantica* subsp. *atlantica*. The Flow Cytometry (FCM) method was a rapid tool to guide us and an efficient technique to check the genome size variability. The finding showed no significant difference of genome size between the individuals and qualified *P. atlantica* as having a very small genome with 2C = 1.21±0.02 pg. The current FCM analysis and the chromosome counting confirm the diploidy of *P. atlantica* subsp. *atlantica*. In fact, in the current study we counted 30 chromosomes (=2n) in prometaphase and we showed the separation of satellites from two chromosomes through the secondary constrictions. We suppose that this observation justified previous notes about previous errors done in the counting of *Pistacia* ssp. chromosomes. At the end of the current study, it was important to assess genetic diversity in *P. atlantica* subsp. *atlantica*. It was the first essay of genetic characterization of the subspecies through microsatellite markers. The SSRs molecular markers used allowed us to estimate the overall genetic diversity and genetic fingerprinting in natural populations of *P. atlantica* subsp. *atlantica* in Northwest Algeria. In the UPGMA analysis, no significant correlation was observed between geographic distance and genetic diversity. Thus, the geographic distance seems to do not be an obstacle to gene dispersal between populations of *P. atlantica* subsp. *atlantica* in Northwest Algeria. Many factors can explain this finding such the edibility and lightness of seeds, the dioecy, and the anemophily. Nevertheless, we can observe genotypes from the same site closely related to each other. It was interesting to see the sites AF2 and OM closely genetically related knowing they are closely geographically located and they shared the characteristics of large leaves and leaflets and of the very dark green leaf colour according to the morphological characterization. In addition, the sites SBA and 3R were genetically closely related and they were characterized by germacrene D and *E*-caryophyllene as major compounds in the chemical composition of essential oils. To

confirm the available pattern it is necessary to use more SSR markers and more number of accessions. More genetic diversity studies are required to learn about the structure and the evolution of natural *P. atlantica* subsp. *atlantica* in Algeria. It would be premature to comment precisely, in this study, about the structure of the natural populations of *P. atlantica*. Nevertheless, the overall findings and mainly the divergences observed in the morphological, the chemical and the genetic characterizations tend to discount the hypothesis to have other subspecies in Algeria. It would be rather one taxon with significant variability originated from various factors. Our results suggest that under climatic variation, the phenotypic plasticity and the genetic diversity of *P. atlantica* are likely to play a crucial role in allowing the species to persist in its environment. The genetic diversity probably originated from long-range dispersal and the mosaic population structure is likely a result of dispersal of pollen or seeds.

This preliminary work was done on a relatively small area if we take into account the vast pistachio range in Algeria or even in the Maghreb, since the issue of morphological variability was also raised by colleagues in Morocco (Yaaqobi *et al*., 2009). It would be interesting to compare these results to larger areas with different disturbances, under different environmental conditions and using more molecular markers in order to demonstrate the process of the evolution and the structuring of Atlas pistachio natural populations. It is very difficult to separate the ecological effects caused by climatic and atmospheric changes from those caused by changes of the mode of land use and the considerable anthropogenic pressure. Atlas Pistachio which was able to develop special strategies to persist in the environment may nevertheless show a weakness against anthropogenic disasters. A critical task is to map the spatial structure of the genetic variation and to relate this to its robustness to further habitat transformation. If this is the role of the researches, several conservation methods of both *in situ* and *ex situ* need to be applied to have a successful conservation strategy and to optimize conservation management plans for the species. *P. atlantica* is qualified as 'multi-use species'; moreover the trees constitute a particular forest formations mainly in the Algerian steppes and in the desert. But also in the North, they constitute a dense formation where the forest species are not yet installed. We believe that both of these plant formations are essential in ecosystem composition in Algeria and both of them should be preserved.

The results of the current work and those of others conducted in other Algerian universities seem promising and could be combined and exploited to develop a mapping based on the macro/micro-morphological, chemical and genetic characterization and also a mapping of valuation fields and pathways of *P. atlantica*. This mapping will avoid any irrational exploitation or any genetic

pollution in the case of *in-situ* and *ex-situ* conservation programs. In this way, we believe that it is very important to pay attention to seed origins in 'ecological restoration and biological conservation purposes and programs'. It is wise for example that seeds from every population or at least from every area should be used to produce seedlings for the restoration of the same population. Conservation and protection of this species require multidisciplinary and multi-sectorial actions. It is time to engage all actors in society for organized and standardized strategies. It would be important to protect the populations of any particularity (chemical, genetic or morphological) and populations under perturbations generating regeneration problems. We regret to mention here that we investigated some populations where only 5 individuals remain and where downsizing populations may lead to a loss in genetic diversity. The irreversible loss of alleles is of great concern because it is the basic functional unit of heredity and the primary source of variation in appearance, characteristics and behavior of plants. This is why, it would be appropriate to establish some protected areas, strategic reserves and/or Biosphere reserves according to the characteristics and the potentialities of the populations. For academic and research institutions, it is imperative to create official herbarium and official seed, pollen and gene banks. The *ex-situ* conservation is an important complementary approach to the *in-situ* conservation.

At the end of this thesis 'I' personally regret to finish it with a bitter feeling because of the loss of two huge trees in my study area. But more distressing is that the legislative texts relating to the protection of plants (knowing that the Atlas pistachio is among the list of protected species in Algeria (***Journal official de la République Algérienne Démocratique et Populaire, N°3: Mercredi 24 Safar 1433, Correspondant au 18 janvier 2012***) are not sufficient to protect the spontaneous flora in Algeria. 'I' believe that they require a serious revision and a strict application. Protection against the loss of genetic diversity is urgently recommended.

WHEN IGNORANCE ACTS

REFERENCES


FLORA ATLANTICA,
SIVE
HISTORIA PLANTARUM,
QUÆ IN ATLANTE, AGRO TUNETANO
ET ALGERIENSI CRESCUNT.
AUCTORE RENATO DESFONTAINES,
TOMUS SECUNDUS.
PARISIIS,
Typis C. PANCKOUCKE.

REFERENCES

[A]

*/Aboura R. 2006. Comparaison phyto-ecologique des Atriplexaies situées au nord et au sud de Tlemcen. Mem. Mag. Univ Tlemcen. 210p+ Annexes.

*/Adams RP. 1998. The leaf essential oils and chemotaxonomy of *Juniperus* sect. *Juniperus. Biochemical and Systematic Ecology*, 26: 637- 645.

*/Adams RP. 2004. Identification of Essential Oils Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, IL, USA.

*/Adanson M. 1763. Familles des plantes. Vincent, Paris, France.

*/Addisalem AB, Esselink GD, Bongers F and Smulders MJM. 2015. Genomic sequencing and microsatellite marker development for *Boswellia papyrifera*, an economically important but threatened tree native to dry tropical forests. *AoB Plants* 7: plu086; doi:10.1093/aobpla/plu086

*****/Adra H. 2011. (in arabic) Somayd: قرية سورية ما زال أهلها يعصرون زيت ثمار شجرة البطم. www.Awsat.com (Copyright: 1978-2013 © H H saudi Research and Marketing LTD). (webmaster: http://aawsat.com/details.asp?section=67&article=616350&issueno=11820#.UnwLB3BFX7E, accessed 07/11/2013.

*/AFNOR 1998 (Association Française de Normalisation) NF T 75-006. in: A.F.D. normalisation (Ed.), Recueil de Normes Française, Huiles essentielle.

*/Agoumi A. 2003. Vulnerability of North African countries to climatic changes adaptation and implementation strategies for climate change, in Developing perspectives on climate change: Issues and analysis from developing countries and countries with economies in transition, IISD/Climate change knowledge network, www.cckn.net//pdf/north_africa.pdf.

*/Agrawal AA. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science's compass*, 294: 321-326.

*/Aime S. 1991. Etude écologique de la transition entre les bioclimats subhumides, semi arides et arides dans l'étage thermoméditerraneen du Tell Oranais (Algérie nord occidentale). Thès. d'état. Univ. Aix – Marseille 3. 190 p.

*/Ait Said S, Fernandez C, Greff S, Derridj A, Gauquelin T and Mevy JP. 2011a. Inter-population variability of leaf morphoanatomical and terpenoid patterns of *Pistacia atlantica* Desf. ssp. *atlantica* growing along an aridity gradient in Algeria. *Flora* 206 (4): 397-405.

*/Ait Said S, Fernandez C, Greff S, Torre F, Derridj A, Gauquelin T and Mevy JP. 2011b. Inter-population variability of terpenoid composition in leaves of *Pistacia lentiscus* L. from Algeria: A chemoecological approach. *Molecules*, 16: 2646-2657.

*/Albaladejo RG, Sebastiani F, Aparicio A, Buonamici A, González-Martínez SC and Vendramin GG. 2008. Development and characterization of eight polymorphic microsatellite loci from *Pistacia lentiscus* L. (Anacardiaceae). *Molecular Ecology Resources* 8, 904–906 doi: 10.1111/j.1755-0998.2008.02110.x

*/Alcaraz C. 1969. Principaux types de successions géographiques concernant le pin d'Alep et ses principales espèces- climax concurrentes dans le Tell oranais. *Bulletin de la Société d'Histoire Naturelle d'Afrique du Nord*, 60 :103-115.

*/Al Dassouki M. 2011. (in arabic) الأشجار تزين المعابد الفرعونية في اليوم العالمي للبيئة - بوابة الأهرام I. (webmaster: http://gate.ahram.org.eg/NewsContentPrint/5/35/79997.aspx accessed 09/11/2013).

*/Aliyu OM. 2012. Development of the flow cytometric protocol for ploidy analysis and determination of relative nuclear DNA content in cashew (*Anacardium occidentale* Linn.). *American Journal of Biochemistry and Molecular Biology*, 2: 200-215. doi: 10.3923/ajbmb.2012.200.215

*/Alpert P, Ben-Gai T, Baharad A, Benjamini Y, Yekutieli D, Colacino M, Diodato L, Ramis C, Homar V, Romero R, Michaelides S, and Manes A. 2002. The parodoxical increase of mediterranean extreme daily rainfall in spite of decrease in total values. *Geophysical Research Letters*, 29 (0), doi :10.1029/2001GL013554.

*/Al-Saghir MG. 2006. Phylogenetic Analysis of the Genus *Pistacia* (Anacardiaceae). PhD thes, Virginia Polytechnic Institute and State University. 112p.

*/Al-Saghir MG. 2010a. "Phylogenetic analysis of the genus *Pistacia* L. (Anacardiaceae) based on morphological data, *Asian Journal of Plant Sciences*, 9 (1): 28-35.

*/Al-Saghir MG. 2010b. Perspective on chromosome numbers in the genus *Psiatcia* L. (Anacardiaceae). *International Journal of Plant Breeding Genetic*, 4 (3): 153-157.

*/Al-Saghir MG, Abu Baker S and Pusok R. 2014. Effective method to resolve the chromosome numbers in *Pistacia* species (Anacardiaceae). *American Journal of Plant Science*, 5: 2913-2916. http://dx.doi.org/10.4236/ajps.2014.520306

*/Al-Saghir MG and Porter DM. 2006. Random Amplified Polymorphic DNA (RAPD) Study of *Pistacia* Species (Anacardiaceae). *Asian Journal of Plant Science*, 5 (6) :1002-1006.

*/Al-Saghir MG and Porter DM. 2012. Taxonomic Revision of the Genus *Pistacia* L. (Anacardiaceae). *American Journal of Plant Science*, 3: 12-32.

*/Al-Saghir MG, Porter DN and Nilsen ET. 2006. Leaf anatomy of *Pistacia* species (Anacardiaceae). *Journal of Biological Science*, 6 (2): 242-244.

*/Al Yafi J. 1978. New characters differentiating *Pistacia atlantica* subspecies. *Candollea*, 33 (2): 201-208.

*/Al Yafi J .1979. Approches systématique et écologique du genre *Pistacia* L. dans la région méditerranéenne. Thès doc Univ. D'Aix-Marseille St Jérome France.

*/Angot A. 1881. Étude sur le climat de l'Algérie (température, pression barométrique et pluie). *Annales du Bureau Central Météorologique*, Paris (13): 7-36.

*/APG (Angiosperm Phylogeny Group) 1998. An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Garden* 85:531-553.

*/APG (Angiosperm Phylogeny Group) 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowewring plants: APGII. *Botanical Journal of the Linnean Society* 141: 399-436.

*/APG (Angiosperm Phylogeny Group) 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APGIII. *Botanical Journal of the Linnean Society*, 161: 105–121. With 1 figure.

*/Arabnezhad H, Bahar M and Pour AT. 2011. Evaluation of genetic relationships among Iranian pistachios using microsatellite markers developed from *Pistacia khinjuk* Stocks. *Scientia Horticulturae*, 128: 249-254.

*/Appleby N, Edwards D and Batley J. 2009. New technologies for ultra-high throughput genotyping in plants. In Methods in molecular biology, plant genomics. vol. 513. Edited by Gustafson JP, Langridge P, Somers DJ Totowa NJ.: 2011. Humana Press,19–38.

*/Asch VM, Tienderen PH, Holleman LJM and Visser ME. 2007. Predicting adaptation of phenology in response to climate change, an insect herbivore example. *Global Change Biology*, 13 : 1596-1604.

*/Athman A, Tanz SK , Conn VM, Jordans Ch, Mayo GM, Ng WW, Burton RA, Conn SJ and Gilliham M. 2014. Protocol: a fast and simple in situ PCR method for localising gene expression in plant tissue. *Plant Methods* , 10:29, http://www.plantmethods.com/content/10/1/29

*/Ayasse M, Schiestel F, Paulus HF, Lofstedt C, Hansson B and Ibarra F. 2000. Evolution of reproductive strategies in the sexually deceptive orchid species *Ophrys sphegodes*: how does flower-specific variation of odour signals influence reproductive success? *Evolution*, 54: 1995–2006.

*/Ayaz E and Namli S, 2009. The karyotype analysis of *Pistacia vera* L. from Turkey. *Natural Product Research*, 23:9, 866-870, doi: 10.1080/14786410802263675.

[B]

*/Barkoulas M, Galinha C, and Grigg SP. 2007. From genes to shape: regulatory interactions in leaf development. *Current opinion plant biology*, 10:660-6.

*/Barrero AF, Herrador MM, Arteaga JF, Akssira M, Mellouki F, Belgarrabe A, Blàzquez M. 2005. Chemical composition of the essential oils of *Pistacia atlantica* Desf., *Journal of Essential Oil Research*, 17: 52-54.

*/Basha AI. 2006. Assess of genetic diversity of *pistacia* spp. in wild populations and field gene banks from central and west Asia. Doc Thesis**.** Univ. Tuscia Italy.

*/Battandier JA. 1910. Flore d'Algérie: Supplément aux phanérogames. Paris, P. Klincksieck. P 35.

*/Battandier JA and Trabut L. 1888 Flore d'Algérie. Alger : Adolphe Jourdan libraire-éditeur, Paris p191,

*/Battandier JA and Trabut L. 1902. Flore analytique et synoptique de l'Algérie et de la Tunisie. Alger : Vve Giralt imprimeur-éditeur.

*/Bazile-Robert E, Suc JP and Vernet JL. 1980. L'Histoire floristique des régions méditerranéennes depuis le début du Pliocène. *Naturalia Monspeliensia.* 237: 311-312.

*/Beard JS. 1945. The Natural Vegetation of Trinidad, 20. Clarendon Press, Oxford, UK.

*/Beckmann JS and Soller M. 1990. Toward a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellite sites. *Biotechnology*, 8:930-2.

*/Behboodi BS. 2004. *Pistacia atlantica* Desf. 1800 in Iran. FAO-CIHEAM - *Nucis-Newsletter*, 12: 27-29.

*/Behboodi BS. 2005. Ecological distribution study of wild pistachios for selection of roostock . In : Oliveira MM (ed.), Cordeiro V. (ed.). XIII GREMPA Meeting on Almonds and Pistachios. Zaragoza: CIHEAM, *Option Méditerranéenne* 63 : 61-67.

*/Behboodi BS and Ghaffari M. 2005. Pollen morphology and analysis of Iranian wild pistachio. In : Oliveira M.M. (ed.), Cordeiro V. (ed.). XIII GREMPA Meeting on Almonds and Pistachios . Zaragoza : CIHEAM, p. 123-127. (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 63).

*/Beker B, Dafni A, Eisikowitch D and Ravid U. 1989. Volatiles of two chemotypes of Majorana syriaca L. (Labiatae) as olfactory cues for honeybee. *Oecologia*, 79, 446-451.

*/Belhadj S, Derridj A, Aigouy Th, Gers Ch, Gauquelin Th, and Mevy J-Ph. 2007b. Comparative morphology of leaf epidermis in eight populations of Atlas Pistachio (*Pistacia atlantica* Desf., Anacardiaceae). *Microscopy research and technique* 70:837-846.

*/Belhadj S, Derridj A, Auda Y, Gers C, Gauquelin T. 2008. Analyse de la variabilité morphologique chez huit populations spontanées de *Pistacia atlantica* en Algérie. *Botany* 86 : 520-532.

*/Belhadj S, Derridj A, Civeyrel L, Gers Ch, Aigouy Th, Otto Th and Gauquelin Th. 2007a. Pollen morphology and fertility of wild Atlas pistachio (*Pistacia atlantica* Desf., Anacardiaceae). *Grana*, 46 (3): 148-156 doi: 10.1080/00173130701520310

*/Belhamri R. 2005. Extraction des macromolécules pariétales des eaux de presse de betteraves sucrières. Etude de leur composition, de leurs propriété´s physicochimiques et de leur effet sur le processus sucrier. Thes. Doc Université de Reims Champagne. Ardenne p. 245.

*/Belyagoubi-Benhammou N, Belyagoubi L, **El Zerey-Belaskri A** and Atik-Bekkara F. 2014. *In vitro* antioxidant properties of flavonoid fractions from *Pistacia atlantica* Desf. subsp. *atlantica* fruit using five techniques. *Journal of Material and Environmental Sciences*. 6 (4): 1118-1125.

*/Belyea RL and Ricketts RE. 1993. Forages for cattle: New methods of determining energy content and evaluating heat damage. Columbia. MO: Univ. Missouri, Agricultural Publication G3150.

*/Benabadji N and Bouazza M. 2000. Contribution à une étude bioclimatique de la steppe à *Artemisia herba-alba* Asso. dans l'Oranie (Algérie occidentale). *Revue Sècheresse* 11 (2) : 117-125.

*/Benabdallah FZ. 2012. Etude morphologique des feuilles et des fruits du pistachier de l'atlas (*Pistacia atlantica* Desf.) et valorisation des huiles essentielles des feuilles et de l'oléorésine. Mem. Mag. Univ. Mohamed KHEIDER Biskra. Algérie. 60p

*/Benaradj A, Boucherit H, Bouazza M, Hasnaoui O, Aibout F and Baghdadi D. 2014. Diagnostic phyto-écologique du groupement préforestier à Pistacia atlantica Desf. dans l'Atlas saharien oranais (Béchar-Algérie). (Proceedings of 2nd international conference of plant biodiversity' Marrakech (Morocco) march 27-29, p 12).

- */ Benhassaini H. 2004. Contribution à l'étude de l'autoécologie de *Pistacia atlantica* Desf.ssp. *atlantica* et valorisation. Thèse de doctorat en Ecologie Appliquée. Univ. Sidi Bel Abbes.

*/Benhassaini H, Benabderrahmane M and Chikhi K. 2003. Contribution à l'évaluation de l'activité antiseptique de l'oléorésine et des huiles essentielles du pistachier de l'Atlas sur certains souches microbiennes : *Candida albicans* (ATCC 20027), *Candida albican* (ATCC 20032) et *Saccbaromyces cerevisiae*. *Ethnopharamcologia*, n° 30.

*/Benhassaini H, Bendahmane M and Benchalgo N. 2007a. The chemical composition of fruits of*Pistacia atlantica* desf. subsp. *atlantica*from Algeria. *Chemistry of Natural Compounds* 43 (2) : 121-124

*/Benhassaini H, Bendeddouche FZ, Mehdadi Z and Romane A. 2008. GC/MS analysis of the essential oil from the oleoresin of *Pistacia atlantica* Desf. subsp. *atlantica* from Algeria. *Natural Product Communication*, 3: 929-932.

*/Benhassaini H, Mehdadi Z, Hamel L and Belkhodja M. 2007b. Phytoécologie de *Pistacia atlantica* Desf. subsp. *atlantica* dans le Nord-ouest algérien. *Sécheresse* 18 (3) : 199-205.

*/Bennett MD, Bhandol P and Leitch IJ. 2000. Nuclear DNA amounts in angiosperms and their modern uses, 807 new estimates. *Annals of Botany* 86: 859-909

*/Bennett MD and Leitch IJ. 1995. Nuclear DNA amounts in angiosperms. *Annals of Botany* 76: 113-176.

*/Bennett MD and Leitch IJ. 2005. Genome size evolution in plants. – In: Gregory T. (ed.), The evolution of the genome, p. 89-162, Elsevier Academic Press, Amsterdam.

*/Bennett MD and Smith JB. 1976. Nuclear DNA amounts in angiosperms. Philosophical Transactions of the Royal Society of London Series B, *Biological Sciences* 274: 227-274.

*/Benslama M, Andrieu-Ponel V, Guiter F, Reille M, de Beaulieu J-L, Migliore J, Djamali M. 2010. Pollen analysis from two littoral marshes (Bourdim and Garaat El-Ouez) in the El-Kala wet complex (North-East Algeria). Lateglacial and Holocene history of Algerian Vegetation. *Comptes Rendus Biologies* 333: 744-754.

*/Bergmans MEF, Beldman G, Gruppen H and Voragen AGJ. 1996. Optimisation of the selective extraction of glucurono-arabinoxylans from wheat bran: Use of Barium and Calcium hydroxide solutions at elevated temperatures. *Journal of Cereal Science*, 23: 235-245.

*/Bertrand I, Chabbert B, Kurek B and Recous S. 2006. Can the biochemical features and histology of wheat residues explain their decomposition in soil? *Plant and Soil* 281, 291-307.

*/Besancenot JP. 1986. Bioclimatologie méditerranéenne. *Annal de Géographie* 95 (532) : 709-715.

*/Bessam FZ. 2008. Contribution à l'étude de *Stipa tenassicima* L.: Germination Caractérisation de certains polyphénoles et effet d'obtention du caryotype (96 p.). Algérie: Mem. Magis. Université Sidi Bel Abbes.

*/Bhattacharyya B and Johri BM. 1998. Flowering Plants: Taxonomy and Phylogeny. Springer Verlag, Berlin, Germany.

*/Biltekin D, Popescu SP, Suc J-P, Quézel P, MorenoGJ, Yavuz N, Cagatay MN. 2015. Anatolia: a long-time plant refuge area documented by pollen records over the last 23 Million years. Review of Palaeobotany and Palynology, Elsevier, 215:1-22.

*/Biomedical Optical Phase Microscopy and Nanoscopy. 2012. In Shaked NT, Zalevsky Z and Satterwhite LL eds. Academic Press, 432p.

*/Birks HJB and Line JM. 1993. Glacial refugia of European tress, a matter of chance? Disssert. Bot. 196: 283-291.

*/Bochantseva ZP. 1972. Ochislakh Chromosome. Introd. Akkli. Rasteny Akad. Nauk. UZ. SSR, 44-53.

*/Boissier E. 1849. Diagnoses Plantarum Orientalium Novarum. Paris, Typis Marci Ducloux et Cons. Series 1, 2 (9): 1.

*/Borowicz K (1988) Chorology of trees and shrubs in South-West Asia and adjacent regions. Polish Scientific, Warszawa Poznan.

*/Bouazza M and Benabadji N. 2008. Évolution climatique et dynamique des écosystèmes naturels de l'Algérie occidentale. 12°e Congrès de L'AFAS « Changement Climatique et Biodiversité » (22-23 Mai 2008, Paris).

*/Boudouaya M, Benhassaini H, Bendimered-Mouri F-Z, Mothe F, Fournier M. 2015. Évaluation de la durabilité naturelle du bois de *Pistacia atlantica* Desf. du Nord de l'Algérie. Bois et forêts des tropiques, 325 (3) : 49-58.

*/Boudy P. 1950. Economie forestière Nord africaine. Tome II, monographie et traitement des essences forestières. Larousse (edn) Paris 610p

*/Boudy P. 1952. Guide forestier en Afrique du Nord. La Maison Rustique (edn), Paris, p196.

*/Bouzidi MA, Latrèche A, Attaoui I, Mehdadi Z, Benyahia M, Bouguenaya N and Meliani H. 2010. Caractérisation et valorisation des polysaccharides pariétaux d'*Urginea pancration* (Steinh) Phil. de Djebel Tessala (Nord-Ouest Algérien). Les Technologies de Laboratoire, 5(19): 23-29.

*/Bozorgi M, Memariani Z, Mobli M, Surmaghi MHS, Shams-Ardekani MR, and Rahimi R. 2013. Five *Pistacia* species *(P. vera, P. atlantica, P. terebinthus, P. khinjuk, and P. lentiscus):* A Review of Their Traditional Uses, Phytochemistry, and Pharmacology. Hindawi, Publishing Corporation, *The Scientific World Journal* 33 p. http://dx.doi.org/10.1155/2013/219815.

*/Bradshaw AD. 1965. Evolutionary significance ofphenotypic plasticity in plants. *Advances in Genetics*, 13:115-155.

*/Bremer K, Bremer B and Thulin M. 1999. Introduction to Phylogeny and Systematics of Flowering Plants. 5th ed. Uppsala, Sweden, Uppsala University.

*/Brichet M. 1931. Compte rendu du livre des journées de l'arbre fruitier, Alger, 735p.

*/Browicz K. 1988. Chorology of Trees and Shrubs in South-west Asia and Adjacent Regions. Polish Scientific Publications, Poznan Vol. 6.

*/Brown R. 1818. Observations, systematical and geographical, on Professor Christian Smith's collection of plants from the vicinity of the River Congo. In J.H. Tuckey, Narrative of an expedition to explore the River Zaire. London, 420-485.

*/Bruns-Balogh P. 1991. Guide *Desfontaines* herbarium. Guide to the microform collection IDC number 6210.

*/Bryant JP, Provenza FD, Pastor J, Reichardt PB, Clausen TP and du Toit JT. 1991. Interactions between woody plants and browsing mammals mediated by secondary metabolites. *Annual Revue of Ecology and Systematic*, 22, 431-446.

*/Bunge, A. 1835. Mémoires des savantes étrangers de l'academié de Saint - *Pétersbourg*. 2: 89.

[C]

*/Carmello-Guerreiro SM and Sartori Paoli AA. 2002. Ontogeny and Structure of the Pericarp of *Schinus terebinthifolius* Raddi (Anacardiaceae). *Brazilian Archives of Biology and Technology*, 45 (1): 73-79. http://dx.doi.org/10.1590/S1516-89132002000100012

*/Carpita NC and Gibeaut DM. 1993 Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, 3(1):1-30.

*/Carpita N and McCann M. 2000. The cell wall. In Buchanan B, Gruissem W and Jones R (Eds.), Biochemistry and molecular biology of plants, In: American Society of Plant Physiologists, 52 - 108. Rockville.

*/Catrice O, Coba de la Peña T and Brown SC. 2006. Applications en biologie végétale : contraintes, succès, espoirs. Chapitre 12 dans : « La cytométrie en flux », X. Ronot, D. Grunwald, J-F. Mayol, J. Boutonnat, (eds), Tec & Doc - Lavoisier, Paris, pp 235-253.

*/Ceballos L and Ortuño F. 1976. Estudio sobre la vegetación y flora forestal de las Canarias Occidentales. Excmo. Cabildo Insular, Santa Cruz de Tenerife p 354.

*/Cerovic ZG, Samson G, Morales F, Tremblay N and Moya I. 1999. Ultraviolet-induced fluorescence for plant monitoring: present state and prospects. *Agronomie*, 19 : 543-578.

*/Chanda SK, Hirst EL, Jones JKN and Percival EGV. 1950. The constitution of xylan from esparto grass (*Stipa tenacissima* L.) *Journal of the Chemical Society*, 1289-1297

*/Charnay L. 2001. Précision de l'écologie des essences forestières dans les Alpes à l'aide de données spatialisées. Mém. de 3e année FIF, ENGREF, Nancy : 55 p.

*/Chase MW, Soltis DE, Olmstead RG, Morgan D, Les DH, Mishler BD, Duvall MR, Price RA, Hills HG, Qiu Y, Dron KA, Rettig JH, Conti E, Palmer JD, Manhart JR, Sytsma KJ, Michaels HJ, Kress WJ, Karol KG, Clark WD, Hedrén M, Gaut BS, Jansen RK, Kim K, Wimpee CF, Smith JF, Furnier GR, Strauss SH, Xiang Q, Plunkett GM, Soltis PS, Swensen SM, Williams SE, Gadek PA, Quinn CJ, Eguiarte LE, Golenberg E, Learn GH, Graham JR, Barrett SCH, Dayanandan S, and Albert VA. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene rbcL. *Annals of the Missouri Botanical Garden* 80: 528-580.

*/Chenost M and Kayouli C. 1997. Utilisation des fourrages grossiers en régions chaudes. Etude FAO Production et sante´ Animales (p. 135). Rome: Ed FAO.

*/Chenoune K. 2005. La flore et la végétation du Hoggar. Bois For Trop 284 (2): 79-83

*/Christensen J, Carter T and Giorgi F. 2002. Prudence employs new methods to assess European climate change, EOS, 83, 147.

*/Chung AM, Staub JE, Chen JF. 2006. Molecular phylogeny of *Cucumis* species as revealed by consensus chloroplast SSR marker length and sequence variation. Genome 49: 219-229.

*/Cindy L, Wedig E, Jaster H and Kenneth J. 1987. Hemicelluloses monosaccharide composition and *in vitro* disappearance of orchard gross and Alfalfa hay. *Journal of Agricultural and Food Chemistry*, 35, 214-218.

*/Claessens H and Thibaut A. 1995. Le Point sur les facteurs écologiques de production du Douglas dans le sud du sillon Sambre-et-Meuse. *Silva Belgica*, 102 (3) : 7-14.

*/Cleland RE. 1977. The control of cell enlargement. *Symposia of the Society for Experimental Biology*, 31, 101-115.

*/Close DC and McArthur C. 2002. Rethinking the role of many plant phenolics - protection from photodamage not herbivores? *Oikos* 99: 166-172.

*/Connolly JD and Hill RA. 1991. Dictionary of Terpenoids. Chapman and Hall, London.

*/Connolly JD and Hill RA. 2005. Triterpenoids. Natural Product Reports, 22: 487-503.

*/Couladis M, *Özcan* M, Tzakou O, and Akgül A. 2003. Comparative essential oil composition of various parts of the turpentine tree (*Pistacia terebinthus* L.) growing wild in Turkey, *Journal of the Science of Food and Agriculture*, 83(2): 136 - 138.

*/Council of Europe, 1997. European Pharmacopoeia, 3rd ed, Strasbourg.

*/Cour P and Duzer D. 1976. Persistance d'un climat hyper-aride au Sahara Central et Méridional au cours de l'Holocène. *Revue de Géographie Physique et de Géologie Dynamique*, 8 (2-3): 175-198.

*/Cowling RM and Campbell BM. 1980. Convergence in vegetation structure in the Mediterranean communities of California, Chile and South Africa. *Vegetatio* 43, 191-197.

*/Cronquist A. 1968. The evolution and classification of flowering plants. Nelson. London.

*/Cronquist A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York, New York , USA.

*/Cronquist A. 1988. The evolution and classification of flowering plants. 2nd ed., New York Botanical Garden, New York, 555 pages. QK 495 A1C75.

[D]

*/Daget, P. 1977. Le bioclimat méditerranéen : caractères généraux, modes de caractérisation ? *Végétation* 34: 1-20.

*/Daget, P. 1980. Un élément actuel de la caractérisation du monde méditerranéen; Le climat. Coll. Fond. L. Emberger sur la mise en place, l'évolution et la caractérisation de la flore et de la végétation circuméditerranéenne. Montpellier, 9, 10/04, 101-120.

*/Dahlgren RMT. 1980. A revised system of classification of the angiosperms. *Botanical Journal of the Linnaean Society* 80: 91-124.

*/Dahmani W. 2011. Etude de la variabilité du pistachier de l'Atlas (*Pistacia atlantica* Desf.) dans les zones steppiques de la région de Tiaret. Mém. Magister Univ. Tiaret. Algérie. 130p.

*/Dangi RS, Lagu MD, Choudhary LB, Ranjekar PK and Gupta VS. Assessment of genetic diversity in *Trigonella foenum-graecum* and *Trigonella caerulea* using ISSR and RAPD markers. *BMC Plant Biology* 2004, **4**:13 doi:10.1186/1471-2229-4-13

*/Debrach J .1953. Notes sur les climats du Maroc occidental. *Maroc oriental*, 32 (242) : 1122-1134.

*/De Candolle A. 1825. Prodromus systematis naturalis regni vegetabilis. Paris, Treuttel & Würtz. 2: 61-92.

*/De Candolle A. 1928. Origines des plantes cultivées. Paris : Germer Baillière [English translation 1884. Origin of cultivated plants. London : Kegan Paul, Trench].

*/De Candolle C. 1868. Théorie de la feuille. Archives des Sciences Physiques et Naturelles (Bibliott. universelle), 32, 32-64.

*/Degenhardt J, Köllner TG and Gershenzon J. 2009. Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. *Phytochemistry* 70:1621–1637. doi: 10.1016/j.phytochem.2009.07.030

*/De Jong G. 2005. Evolution of phenotypic plasticity: patterns of plasticity and the emergence of ecotypes. *New Phytology* 166:101-117.

*/De Jussieu AL. 1789. *Genera plantarum secundum ordines naturales disposita*. Paris: Herrissant & Barrois.

*/De Lamarck J-B. 1804. Encyclopédie méthodique : botanique ; H. Agasse, Paris, T5, p350. [6] GRIN database, USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network, Beltsville, Maryland, USA (2003). Available online at: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl/101771.

*/Delseny M., Laroche M., Penon P., 1983. Detection of sequences with Z-DNA forming potential in higher plants. *Biochemical and Biophysical Research Communications*, *116*: 113–120.

*/De Martonne E. 1926. Aréisme et indice d'aridité. *Compte Rendu de l'Académie des Sciences*, 181: 1395-1398.

*/Demetzos C, Anastasaki T and Perdetzoglou D. 2002. A chemometric interpopulation study of the essential oil of *Cistus creticus* L. growing in Crete (Greece). Z. *Naturforsch*. 57c, 89–94.

*/Demiral I, Atilgan NG and Sensoz S. 2009. Production of biofuel from soft shell of pistachio (*Pistacia vera* L.). *Chemical Engineering Communications*, 196, 104-115.

*/Dengler N and Kang J. 2001.Vascular patterning and leaf shape. *Current opinion plant biology*, 4: 50-6.

*/Desfontaines RL. 1798. *Flora atlantica*; *sive historia platarium quae in atlante, agro Tunetano et Algeriensi crescent*. V1 Paris, L. G. Desgranges.

*/Desfontaine RL. 1799. *Flora atlantica :sive historia plantarum quae in Atlante, agro tunetano et algeriensi crescunt /auctore Renato Desfontaines*.VII. Parisiis : L.G. Desgranges, 2 : p 364.

*/Desfontaine RL. 1800. *Flora atlantica :sive historia plantarum quae in Atlante, agro tunetano et algeriensi crescunt /auctore Renato Desfontaines*.TI, TII. Parisiis : L.G. Desgranges, (TI : Preface ; TII p 364).

*/Dewick PM. 1999. The biosynthesis of C-5-C-25 terpenoid compounds. *Natural Product Reports*, 16, 97-130.

*/DGF Direction Générale des Forêts. 2015. Etude portant sur le diagnostic écologique des peuplements du Pistachier de l'Atlas et proposition d'un plan d'action pour leur réhabilitation et leur extension. Présentation de la phase 1 de l'étude – Siège de la DGF (Alger, Algérie).

*/D'Hondt L, Hofte M, VanBockstaele E and Leus L. 2011. Applications of flow cytometry in plant pathology for genome size determination, detection and physiological status. *Molecular Plant Pathology*, 12, 815–828.doi:10.1111/j.1364- 3703.2011.00711.x

*/Doležel J, Sgorbati S and Lucretti S. 1992. Comparison of three DNA fluorochromes for flow-cytometric estimation of nuclear DNA content in plants. *Physiologia Plantarum* 85, 625-631.

*/Doležel J. and Bartoš J. 2005. Plant DNA flow cytometry and estimation of nuclear genome size. *Annuals of Botany*, 95: 99-110.

*/Doležel J, Greilhuber J and Suda J. 2007. Flow cytometry with plants: an overview. – In: Doležel J, Greilhuber J and Suda J. (eds), Flow cytometry with plant cells, 41-65, Wiley-VCH, Weinheim.

*/Dong J, Ma X, Wei Q, Peng S and Zhang S. 2011. Effects of growing locations on the contents of secondary metabolites in the leaves of four selected superior clones of *Eucommia ulmoides*. *Industrial Crops* and *Products* 34, 1607-1614.

*/Douglas DA. 1981. The balance between vegetative and sexual reproduction of *Mimulus primuloides* (Scropholariaceae) at different altitudes in California. *Journal of Ecology* 69: 295-310.

*/Doyle JJ and Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*. 19, 11-15.

[E]

*/Edwards A, Civitello A, Hammond HA and Caskey CT. 1991. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *American Journal of Human Genetics*, 49, 746-756.

*/Edwards WN and Wonnacott FM. 1935. Anacardiaceae. Fossilium catalogus II Plantae. Berlin, W. Junk.

*/Ehlers B and Thompson JD. 2004. Do co-occuring plant species adapt to one another? The response of *Bromus erectus* to the presence of different *Thymus vulgaris* chemotypes. *Oecologia*, 141: 511-518.

*/Eichler AW. 1875-1878. Bluthendiagramme construirirt und erlautert, vol. 2. Wilhelm Engelmann, Leipzig, Germany.

*/Eig A. 1931-1932. Les éléments et les groups phytogéographiques auxiliaires dans la flore palestinienne. 2 Parts, Feddes. *Rep. Spec. Nov. Reg. Veg. Beih*, 63 : 1-121, 1-120.

*/El Bouhissi M. 2011. Etude de *Asphodelus microcarpus* Salzm. et Viv. du Mont de Tessala (Algérie Occidentale): Histologie, Autoécologie et Dosage des Polysaccharides pariétaux, Mem. Magis. Biol. Université Sidi Bel Abbes. Algérie 134 p.

*/El-Hamouti N, Lamb HF, Fontes JC, Gasse F. 1991. Changements hydroclimatiques abrupts dans le moyen atlas marocain depuis le dernier maximum glaciaire, *Comptes Rendus de l'Académie des Sciences*, Paris, Ser. II 313: 259-265.

*/El Hamrouni A and Sarson M. 1974. Valeur alimentaire de certaines plantes spontanées ou introduites en Tunisie. Institue National des Recherches Forestières, *Notes de recherche*, 2, 12 pp.

*/Elizabeth H. 2008. (http://www.pfaf.org/user/Plant.aspx?LatinName=Pistacia+atlantica accessed on 12/11/2014).

*/El-Oqlah AA. 1996. Biosystematic research on the genus *Pistacia* in Jordan. In: Padulosi S, Caruso T, Barone E (eds) Taxonomy, distribution, conservation and uses of *Pistacia* genetic resources. IPGRI, Palermo, Italy, pp 12–19.

*/**El Zerey-Belaskri A.** (data not published, in french). Contribution à l'étude biométrique des fruits de l'espèce *Pistacia atlantica* Desf : (régions de : Sfisef, Mestpha Ben Brahim et Ben Badis). Travaux de recherche pour 'Laboratoire de Recherche 'Biodiversité Végétale : Conservation & Valorisation'

*/**El Zerey-Belaskri A.** (data not published, in french). Enquête ethnobotanique sur le pistachier de pistachier de l'Atlas. Travaux de recherche pour 'Laboratoire de Recherche Biodiversité Végétale : Conservation & Valorisation'

*/**El Zerey-Belaskri A** and Benhassaini H. 2015. Morphological leaf variability in natural populations of *Pistacia atlantica* Desf. subsp. *atlantica* along climatic gradient: new features to update *Pistacia atlantica* subsp. *atlantica* key. *International Journal of Biometeorology*, doi 10.1007/s00484-015-1052-4.

*/**El Zerey-Belaskri A**, Benhassaini H, Naimi W, Rahoui S. 2013. Cellulosic and hemicellulosic fractions dosage of *Pistacia atlantica* Desf. subsp. *atlantica* leaves in western Algeria. *Natural Product Research*, 27 (19) : 1757-1763.

*/**El Zerey-Belaskri A**, Cavaleiro C, Romane A, Salgueiro L and Benhassaini H. 2014b. Intraspecific chemical variability of the essential oils of *Pistacia atlantica* Desf. subsp. *atlantica* from the north-west region of Algeria (Proceeding of the 5th International congres of aromatic and medicinal plants (CIPAM), Zarziz Tunisia, march 17-20 2014.

*/**El Zerey-Belaskri A**, Lalout JFZ, Oughilas A. et Benhassaini H. 2014c. Effet de la conservation des drupes de *Pistacia atlantica* Desf. *subsp. atlantica* (région d'E Bayadh) sur les propriétés physico-

chimiques de l'huile lourde et perspectives de son utilisation comme huile conventionnelle. (Proceeding of the 5th International congres of aromatic and medicinal plants (CIPAM), Zarziz Tunisia, march 17-20 2014.

*/**El Zerey-Belaskri A**, Hakemi Z, Saidi B, Bouajaj S, Ghalbane I, Benhammou N, Romane A, Benhassaini H. 2014c. Evaluation de l'activite antioxydante des extraits méthanoliques et des alcaloïdes totaux des feuilles de *Pistacia atlantica* Desf. subsp. *atlantica (*Algerie Nord-occidentale) (Proceeding of the 5th International congres of aromatic and medicinal plants (CIPAM), Zarziz Tunisia, march 17-20 2014.

*/**El Zerey-Belaskri A**, Khelifi Y, Benhassaini H. 2014a. The adaptive behaviour of spontaneous populations of *Pistacia atlantica* Desf. subsp. *atlantica* towards bioclimate range in Algeria. (Proceedings of 2nd international conference of plant biodiversity' Marrakech (Morocco) march 27-29, p10, 11).

*/Emberger L. 1930. La végétation de la région méditerranéenne, essai d'une classification des groupements végétaux. *Revue Générale de Botanique* , 42 : 641-662 et 705-721.

*/Emberger L. 1941. Les limites de l'aire de végétation méditerranéenne en France. *Bulletin de la Société d'Histoire Naturelle de Toulouse,* 1943, 78, 3, 159-180.

*/Emberger L. 1942. Un projet de classification des climats du point de vue phytogéographique. *Bulletin de la Société d'Histoire Naturelle de Toulouse,* 77 : 97-124.

*/Emberger L. 1955. Une classification biogéographique des climats. Travaux du Laboratoire de Botanique et de Zoologie de la Faculté des Sciences (Trav Lab Bot Zool Fac Sci Serv Bot), Montpellier, 7 : 3-43.

*/Endlicher SL. 1836-1840. Genera plantarum. Wien.

*/Engler A . 1876 . Anacardiaceae. *In* C. F. P. Martius (eds.), Flora Brasiliensis, 12 (2): 367-418. F. Fleischer, Munchen and Leipzig, Germany.

*/Engler A. 1883. *Anacardiaceae. In*: De Candolle ALP (ed.), *Monographiae phanerogamarum*.

*/Engler A. 1892. Anacardiaceae. *In* A. Engler and K. Prantl (eds.), Die natürlichen Pfl anzenfamilien, 3 (5): 138 – 178. W. Engelmann, Leipzig, Germany.

*/Engler A. 1896. Anacardiaceae. *In:* Engler A and Prantl K (eds.) *De natürlichen Pflantzenfamilien*, T. 3, Abt. 5. Wilhelm Engelmann, Leipzig, pp. 138-178.

*/Erdtman G. 1971. Pollen Morphology and Plant Taxonomy. Hafner Publishing Company, New York, USA.

*/Eur. Ph. 2008. European Pharmacopoeia General Monograph "Essential oils" 01/2008:2 89.

*/Evans WC. 2002. Trease and Evans' pharmacognosy 13th edn, Baillière Tindall, London.

*/Excoffier L, Laval G and Schneider S. 2005. Arlequin (ver. 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* . Online 1: 47-50.

[F]

*/Fahna A and Evert RF. 1974. Ultrastructure of the secretory ducts of *Rhus glabra* L. *American Journal of Botny*, 61 (1): 1-14.

*/Fasihi-Harandi OF and Ghaffari M. 2001. Chromosomal Studies on Pistachio (*Pistacia vera* L.) from Iran. *Cahiers Options Méditerranéennes*, 56, 35-40.

*/Fauquette S, Suc J-P, Guiot J, Diniz F, Feddi N, Zheng Z, Bessais E and Drivaliari A. 1999. Climate and biomes in the West Mediterranean area during the Pliocene. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 152: 15-36.

*/Fernandes ES, Passos GF, Medeiros Rda Cunha FM, Ferreira J, Campos MM, Pianowski LF and Calixto JB. 2007. Anti-inflammatory effects of compounds alpha-humulene and (-)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. *European Journal of Pharmacology*, 569: 228-236.

*/Fischer FG and Meyer A. 1838. Enumeratio plantarum quas in Provincia Talysch collegit. *Bulletin de la société impériale des naturalistes de Moscow*, 4: 338-339.

*/Franchet A. 1886. *Plantae Yunnanenses*. *Bulletin de la Société Botanique de France*, Paris, 33: 467.

*/Frenzel B. 1979. L'homme comme facteur écologique en Europe, *Bulletin de l'Association Française des Etudes Quaternaires*, 4, 191-199.

*/Frey-Wyssling A. 1969. The ultrastructure and biogenesis of native cellulose. *Fortschritte der Chemie Organischer Naturstoffe*, 27, 1-30.

[G]

*/Gadek PA, Fernando ES, Quinn CJ, Hoot SB, Terrazas T, Sheahan MC and Chase MW. 1996. Sapindales: Molecular delimitation and infraordinal groups. *American Journal of Botany*, 83: 802-811.

*/Galbraith DW, Harkins KR, Maddox JM, Ayres NM, Sharma DP and Firoozabady E. 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. *Science*, 220: 1049-1051.

*/Gardeli C, Vassiliki P, Athanasios M, Kibouris T and Komaitis M. 2008. Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: evaluation of antioxidant capacity of methanolic extracts. *Food Chemistry*, 107: 1120-1130.

*/Ge G-B, Zhang Y-Y, Hao D-C, Hu Y, Luan H-W, Liu X-B, He Y-Q, Wang Z-T and Yang L. 2008. Chemotaxonomic study of medicinal *Taxus* species with fingerprint and multivariate analysis. *Planta Medica*, 74, 773-779.

*/Geesink AJ, Leeuwenberg M, Ridsdale CE and Veldkamp JF. 1981. Thonner's Analytical Key to the Families of Flowering Plants. University of Leiden Press, Leiden, Netherlands.

*/ Ghaffari SM, Behboodi BS and Shabazaz M. 2005. Chromosome variation in *Pistacia* genus. *In*: Oliveira MM and Cordeiro V. (eds.). XIII GREMPA Meeting on Almonds and Pistachios . Zaragoza : CIHEAM, 347 -354 (*Options Méditerranéennes* : Série A. Séminaires Méditerranéens; n. 63).

*/Ghaffari SM and Fasihi-Harandi O. 2002. Chromosome counts and assessment of two heterochromatic chromosomes in some species of *Pistacia* L. *Acta Horticulturae*, 591: 389-393.

*/Gharzouli R. 1977. Essai de détermination de gradient altitudinaux pluviométriques et thermiques en zone aride : applications bioclimatiques. Mem. D.E.S. :U.S.T.H.B, Alger.

*/Ghelardini C, Galeotti N, Di Cesare Mannelli L, Mazzanti G and Bartolini A. 2001. Local anaesthetic activity of β-caryophyllene, *Il Farmacology*, 56: 387-389.

*/Ghorbel A, Ben Salem-Fnayou A, Chatibi A, Twey M. 1998. Genetic resources of *Pistacia* in Tunisia. *In* Padulosi S, Hadj Hassan A (eds.): Towards a comprehensive documentation and use of *Pistacia* genetic diversity in Central and West Asia, North Africa and Europe. Report of the IPGRI Workshop, 14-17 December: 62-71.

*/Gill BS, Bir SS and Singhal VK. 1984. Cytological Studies in Some Western Himalayan Wood Species II. Polypetalae. *In*: Paliwal GS ed. The Vegetational Wealth of the Himalayas, Puja Publication, Delhi, 497-515.

*/Givnish TJ. 1984. Leaf and canopy adaptations in tropical forests. Physiological Ecology of Plants of the Wet Tropics (Medina E, Mooney HA and Vazquez-Yanes C eds.), 51-84. Dr W. Junk, The Hague, the Netherlands.

*/Golan-Goldhirsh A, Barazani O, Wang ZS, Khadka DK, Saunders JA, Kostiukovsky V, Rowland LJ. 2004. Genetic relationships among Mediterranean *Pistacia* species evaluated by RAPD and AFLP markers. *Plant Systeme Evolution,* 246, 9-18.

*/Gregoriou C. 2001. Collection, conservation and utilization of *Pistacia* genetic resources in Cyprus. In Padulosi S. and A. Hadj-Hassan, eds. Project on Underutilized Mediterranean Species. *Pistacia*: towards a comprehensive documentation of distribution and use of its genetic diversity in Central & West Asia, North Africa and Mediterranean Europe. Report of the IPGRI Workshop, 14-17 December 1998, Irbid, Jordan.

*/Guichoux E, Lagache L, Wagner S, Chaumeil P, Léger P, Lepais O, Lepoittevin C, Malausa T, Revardel E, Salin F and Petit RJ. 2011. Current trends in microsatellite genotyping. *Molecular Ecology Resources*, 11, 591-611.

*/Gundersen A. 1950. Families of dicotyledons. Chronica Botanica Company, Waltham, MA, USA.

*/Guo X and Elston RC. 1999. Linkage information content of polymorphic genetic markers. Human Heredity, 49:112-118.

*/Gupta PK and Varshney RK. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*, 113: 163-85.

*/Godin B, Ghysel F, Agneessens R, Schmit T, Gofflot S, Lamaudière S and Delcarte J. 2010. Détermination de la cellulose, des hémicelluloses, de la lignine et des cendres dans diverses cultures lignocellulosiques dédiées à la production de bioéthanol de deuxième génération (14) 2, numero spécial Base Gembloux. Belgique.

*/Gourine N. Bombara I, Yousfi M and Gaydou EM. 2009. Chemotype investigation for essential oil of leaves of *Pistacia atlantica* Desf. from Algeria. *Natural Product Communication*, 4 (0): 1-6.

*/Gourine N, Bombarda I, Nadjemi B, Yousfi M, Gaydou EM. 2010b. Chemotypes of *Pistacia atlantica* leaf essential oils from Algeria. *Natural Product Communications*, 5(1): 115-120.

*/Gourine N, Yousfi M, Bombarda I, Nadjemi B, Stocker P, Gaydou EM. 2010a. Antioxidant activities and chemical composition of essential oil of *Pistacia atlantica* from Algeria, *Industrial Crops and Products*, 31(2): 203-208.

*/Govindarajan M. 2010. Chemical composition and larvicidal activity of leaf essential oil from *Clausena anisata* (Willd.) Hook. f. ex Benth (*Rutaceae*) against three mosquito species. *Asian Pacific Journal of Tropical Medicine*, 874-877.

*/Graham JH, Freeman DC and Emlen JM. 1993. Antisymmetry, directional symmetry, and dynamic morphogenesis. *Genetica* 89: 121-137.

*/Greilhuber J, Dolezel J, Lysak MA and Bennett MD. 2005. The Origin, Evolution and Proposed Stabilization of the Terms ‘Genome Size’ and ‘C-Value’ to Describe Nuclear DNA Contents. *Annals of Botany* 95: 255-260, doi:10.1093/aob/mci019, available online at www.aob.oupjournals.org.

*/Greilhuber J, Borsch T, Müller K, Worberg A, Porembski S and Barthlott W. 2006. Smallest angiosperm genomes found in *Lentibulariaceae*, with chromosomes of bacterial size. *Plant Biology*, 8: 770-777.

[H]

*/Hallier H. 1908. Über Juliania, veine Terebinthaceen gattung mit cupula, und die wahren Stammeltern der Katzchenblutler. *Beihefte zum Botanischen Centralblatt, Kassel,* 23: 81-265.

*/Harandi FO and Ghaffari M. 2001. Chromosome studies on Pistachio (*Pistacia vera* L.) from Iran. *Options Méditerranéennes*, 56: 35-40.

*/Harche M, Tollier MT, Monties B and Catesson AM. 1991. Caractérisation comparée des constituants (polyosides, lignines et acides phynologiques) des parois cellulaires de trois graminées subdésertiques pérennes: *Stipa tenacissima* L ; *Lygeum spartum* L. et *Aristida pungens* L. *Cellulose Chemistry and Technology*, 25, 11-17.

*/Hardie DC, Gregory TR and Hebert PDN. 2002. "From pixels to picograms: a beginners' guide to genome quantification by Feulgen image analysis densitometry". *Journal of Histochemistry and Cytochemistry* 50 (6): 735–749. doi:10.1177/002215540205000601

*/Hoffmann AA and Sgrò CM. 2011. Climate change and evolutionary adaptation. *Nature*, 470, 479- 485.

*/Hohenacker RF. 1838. *Pistacia mutica* Fisch. & C. A. Mey. *Bulletin de la Société des naturalistes de Moscou*, 11 (4): 338 (Enum. pl. Talysch 102).

*/Hormaza JI. 1995. PCR and the queen of Sheba. FAO - *Nucis-Newsletter*, 4: 15, 16.

*/Hormaza JI. 2002. Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. *Theoretical* and *Applied Genetics*, 104: 321-328.

*/Hormaza JI, Pinney K and Polito VS. 1998. Genetic diversity of pistachio (*Pistacia vera*, Anacardiaceae) germplasm based on randomly amplified polymorphic DNA (RAPD) markers. *Economic Botany*, 52: 78-87.

*/Houghton J, Weatherwax S and Ferrell J. 2006. Breaking the biological barriers to cellulosic ethanol: A joint research agenda. In Houghton J, Weatherwax S and Ferrell J (eds.), A research roadmap resulting from the biomass to biofuels workshop, 7-9 December 2005, Rockville, MA: US Department of Energy.

*/Hsu J. 1983. Late cretaceous and cenozoic vegetation in China, emphasizing their connections with North America. *Annals Missouri Botanical Garden*, 70: 490-508.

*/Huang SF, Chen ZY, Chen SJ, Huang XX, Qi QY and Shi XH. 1986. Plants Chromosome Count (3). *Subtropical Forest Science and Technology*, 4, 50-56.

*/Huang SF, Zhao ZF, Chen ZY, Chen SJ and Huang XX. 1989. Chromosome counts on hundred species and infraspecific taxa. *Acta Botanica Austro Sinica*, 5, 161-176.

*/Hufford KM and Mazer SJ. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology and Evolution*, 18:147-155.

*/Humboldt F, Bonpland AJ and Kunth C. 1824. Nova genera et species plantarum. Paris, Sumptibus librariae graeco-latini-germanicae 7: 22, t. 608.

*/Huntley B. 1991. How plants respond to climate change: migration rates, individualism and the consequences for plant communities. *Annals of Botany*, 67(suppl. 1): 15-22.

*/Hutchinson J. 1926. The families of flowering plants. MacMillan and Co., Limited, London, UK.

*/Hutchinson J. 1973. The families of flowering plants. Clarendon Press, Oxford, UK.

*/Hüsnü K, Başer C and Demirci F. 2007. Chemistry of Essential Oils, *In*: R. Berger (ed.), Flavours and Fragrances, Springer Hannover, pp. 43-86.

[I]

*/Ibn Kathir A. (in arabic) From : al-Bidayah wa'an-Nihayah (The Beginning and the End) [≈ 700 A.H-774A.H], in: al-maaref ed. : Kisas al-Anbiya (The Prophet stories) 2006, Beyrouth p220.

*/Ila HB, Kafkas S and Topaktas M. 2003. Chromosome numbers of four *Pistacia* (Anacardiaceae) species. *Journal of Horticultural Science & Biotechnology*, 78 (1) 35-38

*/Inbar M and Kark S. 2007. Gender-related developmental instability and herbivory of *Pistacia atlantica* across a steep environmental gradient. *Folia geobotanica*, 42: 401-410.

*/Inbar M. 2008. Systematics of *Pistacia*: Insights from specialist parasitic aphids. *Taxon*, 57 (1): 238-242.

*/IPCC (Intergovernmental Panel on Climate change Cambridge). 2001. Climate change 2001. The scientific basis. Contribution of Working Group I to the third assessment report of the IPCC, houghton, ding, griggs, noguer, van der linder, dai, maskell, johnson ed., 881 pp., Cambridge University Press, Cambridge, U.K.

*/IPCC (Intergovernmental Panel on Climate change Cambridge). 2007. Mitigation of climate change: the Working Group III contribution to the Fourth Assessment Report of Intergovernmental Panel on Climate change Cambridge (IPCC): Cambridge University Press. IPCC.

*/IPGRI (International Plant Genetic Resources Institute). 1997. Descriptors for Pistachio (*Pistacia vera* L.). International Plant Genetic Resources Institute, Rome, Italy.

*/IPGRI (International Plant Genetic Resources Institute). 1998. Descriptors for *Pistacia* spp (excluding *P. vera* L.). International Plant Genetic Resources Institute, Rome. Italy.

*/IPNI (International Plant Name Index). 2010. http://www.ipni.org.

*ISO 9235 (International Standard Organization). 1997. International Standard Organization on Essential Oils of the ISO/TC.

[J]

*/Jacob HJ, Lindpaintner K, Lincoln SE, Kusumi K, Bunker RK, Mao YP, Ganten D, Dzau VJ, Lander ES. 1991. Genetic mapping of a gene causing hypertensive rat. *Cell*, 67:213-224.

*/Jeffries TW. 1994. Biodegradation of lignin and hemicelluloses. Section in biochemistry of microbial degradation Ratledge C. (ed.), Dordrecht: Kluwer Academic Publishers, 233-277.

*/Joel D, Marbach I and Mayer AM. 1978. Laccase in Anacardiaceae, *Phytomemistry*, 17: 796.

*/Joshi GV and Gowda CA. 1975. Seasonal variations in chemical composition of *Sargasswn ilicifolium* Grun, sea water. *Indian Journal of Marine Sciences*, 4: 165-168.

*/Jordano P. 1989. Predispersal biology of *Pistacia lentiscus* (Anacardiaceae): Cumulative effects on seed removal by birds. *Oikos*, 55: 357-386.

*/Joret C. 1976. Les plantes dans l'antiquité et au moyen âge; histoire, usages et symbolisme. Slatkine Reprints, Genève. Reprinted from the book first published in 1897-1904.

*/Joulain D and Konig WA. 1998. The Atlas of spectral data of sesquiterpene hydrocarbons. Verlag EB, Hamburg.

*/Judd WS, Campbell CS, Kellogg EA and Stevens P. 2002. Botanique systématique, une approche phylogénétique, De Boeck Université, Paris ISBN 13 : 9782744501234.

*/Jump AS and Penuelas J. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters* 8:1010-1020.

*/Jung CG. 1954. Answer to Job. From vol: 11 of the collected works of Jung CG. Princeton University Press (2012) : 29-31.

[K]

*/Kadi-Bennane S. 2004. Etude biosystématique et évolution adaptative de trois populations de *Pistacia atlantica* Desf.ssp. *atlantica* (Ain Oussera, Messad, Tamanrasset) par le biais du complexe stomatique et d'une approche anatomique. Mem. Magis. I.N.A El Harrach Alger, 112 p.

*/Kafkas , S . 2006 . Phylogenetic analysis of the genus *Pistacia* by AFLP markers. *Plant Systematics and Evolution*, 262 (1) : 113-124

*/Kafkas S, Doğan Y, Zaloğlu S. 2009Phylogenetic analysis in the genus *Pistacia* by simple sequence repeat markers. 5th International Symposium on Pistachios and Almonds. Abstract book, p. 84, October 6–10, , Sanliurfa, Turkey.

*/Kafkas S and Perl-Treves R. 2001. Morphological and molecular phylogeny of *Pistacia* species in Turkey. *Theorical and Applied Genetics*, 102 (6): 908-915.

*/Kafkas S and Perl-Treves R. 2002. Interspecific relationships in *Pistacia* based on RAPD fingerprinting. *Horicultural Science*, 37 (1): 168-171.

*/Kafkas S, Kafkas E and Perl-Treves R. 2002. Morphological diversity and a germplasm survey of three wild *Pistacia* species in Turkey. *Genetic Resources and Crop Evolution*, 261 (49): 261–270

*/Kalendar R, Flavell AJ, Ellis THN, Sjakste T, Moisy C and Schulman AH. 2011. Analysis of plant diversity with retrotransposon-based molecular markers. *Heredity*, 106: 520-30. doi, 10.1038/hdy.2010.93.

*/Karimi HR and Kafkas S. 2011. Genetic relationships among *Pistacia* species studied by SAMPL markers. *Plant Systematics and Evolution*, 297:207–212. doi 10.1007/s00606-011-0508-1

*/Karimi HR, Kafkas S, Zamani Z, Ebadi A, Fatahi Moghadam MR. 2009a. Genetic relationships among *Pistacia* species using AFLP markers. *Plant Systematics and Evolution*, 279 (1): 21-28. doi 10.1007/s00606-008-0117-9.

*/Karimi HR, Zamani Z, Ebadi A and Fatahi MR. 2009b. Morphological diversity of *Pistacia* species in Iran. *Genetic Resources and Crop Evolution*, (56):561-571.

*/Katayama T. 1962. Volatile constituents. Physiology and Biochemistry of Algae. Lewln RA (ed) Academic : 467-473.

*/Katayama T. 1955. Chemical studies on volatile constituents of seaweeds VI. On volatile constituents of *Sargasswn sp. Bulletin* of the *Japanese Society of Scientific Fisheries*, 21: 425-428.

*/Kelkar YD, Strubczewski N, Hile SE, Chiaromonte F, Eckert KA and Makova KD. 2010. What is a microsatellite: a computational and experimental definition based upon repeat mutational behavior at A/T and GT/AC repeats. *Genome Biology and Evolution*, 2, 620–635. doi: 10.1093/gbe/evq046.

*/Kessler S and Sinha N. 2004. Shaping up: the genetic control of leaf shape. *Current Opinion in Plant Biology*,7:65-72.

*/Khatamsaz M. 1978. [*Flora of Iran No. 3: Anacardiaceae*]. Ed. of Organization of Forest and Pasture Research (in Farsi).

*/Kimuram M and Crow J. 1964 The number of alleles that can be maintained in a finite population. *Genetics*, 49 : 725-738.

*/Klemm D, Heublein B. Fink H-P, and Bohn A. 2005. Cellulose: Fascinating Biopolymer and Sustainable Raw Material. *Journal of the Gesellschaft Deutscher Chemiker,* 44 (22): 3358–3393.

*/Kokwaro JO and Gillett JB. 1980. Notes on the Anacardiaceae of eastern Africa. *Kew Bulletin,* 34 : 745 - 760 .

*/Körner C, Neumayer M, Menendez-Riedl SP and Smeetsscheel A. 1989. Functional morphology of mountain plants. *Flora*, 182: 353-383.

*/Kössel A. 1891. Archives of Analytical Physiology, *Physiololgy Abteilung*, 181-186.

[L]

*/Laaidi K. 1997. Bioclimatologie d'une plante xérophile du Sud de la France : le Chêne kermès. Sécheresse, 1 (8): 21-28.

*/Lapie G. 1909. Etude phytogéographique de la kabylie du Djurdjura. Thès. Doc Univ. Paris, 156p.

*/Lapie G. and Maige A. 1914/1924. Flore forestière de l'Algérie. Orlagh *(*Edn). Paris. 358 p. Manuscrit remis le 14 novembre 1924. Dernières épreuves corrigées le 5 décembre 1924.

*/Le Fur D. 1994. La Conservation Des Peintures Murales Des Temples De Karnak. Paris: Recherche sur les civilizations (edn). p60.

*/Le Houérou HN. 1991. La Méditerranée en l'an 2050 : Impacts respectifs d'une éventuelle évolution climatique et de la démographie sur la végétation, les écosystèmes et l'utilisation des terres. La *Météorologie,* 3: 4-37.

*/Le Houérou HN. 1997. Climate, flora and fauna changes in the Sahara over the past 500 million years. *Journal of Arid Environments,* 37: 619-647.

*/Leveau P, Walsh K, Trement F, and Barker G (eds). 1998. Environnemental reconstruction in Mediterranean landscape archeology. In Mediterranean Lanscape Archeology, 2, oxbow Publ, Oxford, pp 145-153.

*/Leitch IJ and Bennett MD. 2007. Genome size and its uses: the impact of flow cytometry. *In*: Doležel J, Greilhuber J and Suda J. (eds), Flow cytometry with plant cells, p. 153-176, Wiley-VCH, Weinheim.

*/Leitch IJ, Chase MW and Bennett MD. 1998. Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. *Annals of Botany*, 82: 85-94.

*/Leitch IJ, Kahandawala I, Suda J, Hanson L, Ingrouille MJ, Chase MW and Fay MF. 2009. Genome size diversity in orchids - consequences and evolution. *Annals of Botany*, 104: 469-481.

*/Levin DA. 1976. The Chemical Defenses of Plants to Pathogens and Herbivores. *Annual Review of Ecology and Systematics*, 7: 121-159, doi: 10.1146/annurev.es.07.110176.001005.

*/Lichtenthaler HK, Ac A, Marek MV, Kalina J, Urban O. 2007. Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. *Plant Physiology and Biochemistry*, 45:577-588.

*/Limane A, Smail-Saadoun N and Gradziel T. 2014. Root architecture of Atlas pistachio in relation to underlying soil properties under arid conditions. African Journal of Agricultural Research, 9(6): 620-626, doi: 10.5897/AJAR2013.7291.

*/Linnaeus C. 1743. *Genera Plantarum*. Paris: Michaelis Antonii David, - *Systema Naturæ*.

*/Linnaeus C. 1753. *Species Plantarum, Laurentii Salvii*, vol. 2, pp. 1025-1026. [Linnaeus 1735-1758].

*/Lindley J. 1830. An introduction to the Natural System of Botany. Longman, Rees, Orme, Brown and Green, London, England.

*/Linhart YB and Grant MC. 1996. Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics*, 27:237-277.

*/Lionello P, Malanotte-Rizzoli P, Boscolo R, Alpert P, Artale V, Li L, Luterbacher J, May W, Trigo R, Tsimplis M, Ulbrich U and Xoplaki E. 2006. The Mediterranean climate: an overview of the main characteristics and issues. *In*: Lionello P, Malanotte-Rizzoli P and Boscolo R. (eds.), Mediterranean Climate Variability. Elsevier, Amsterdam, pp. 1-26.

*/Litt M and Luty JA. 1989. A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetic*, 44: 397-401.

*/Loureiro J, Rodriguez E, Doležel J and Santos C. 2007. Two new nuclear isolation buffers for plant DNA flow cytometry: a test with 37 species. *Annals of Botany* 100: 875-888.

*/Loureiro J, Trávníček P, Rauchová J, Urfus T, Vít P, Štech M, Castro S and Suda J. 2010. The use of flow cytometry in the biosystematics, ecology and population biology of homoploid plants. *Preslia*, 82: 3-21.

*/Louzabi S, Belhadj S And Bouragba-Brague N. 2014. Morphologie des feuilles saines et des feuilles infectées de galles de *Pistacia atlantica* et de *Pistacia terebinthus*. (Proceedings of 2nd international conference of plant biodiversity' Marrakech (Morocco) march 27-29.

*/Ludlow (1983) *In*: Dale JE and Milthorpe FL (eds) The Growth and Functioning of leaves. Cambridge University Press, Cambridge, pp 347-380.

[M]

*/Mabberley DJ. 1997. The Plant Book. Cambridge University Press, Cambridge, UK.

*/Magallon S and Sanderson MJ. 2001. Absolute diversification rates in angiosperm clades. *Evolution*, 55: 1762-1780.

*/Maire R. 1924. Etudes sur la végétation et la flore du grand Atlas et du moyen Atlas marocains. *Société des Sciences Naturelles et Physiques du Moroc*, 220p.

*/Malécot G. 1948. Les Mathématiques de l'Hérédité. Masson et Cie, Paris

*/Maley J. 1980. Les changements climatiques de la fin du Tertiaire en Afrique: leurs conséquences sur l'apparition du Sahara et de sa végétation. In: Williams AJ and Faure H. (eds), The Sahara and the Nile, pp. 63-86. Rotterdam: Balkema AA. 607.

*/Mansf Ency. 2001. Mansfeld's encyclopedia of agricultural and horticultural crops. Volumes 1-6. Author or Editor: Hanelt, P., ed. Url: http://mansfeld.ipk-gatersleben.de/pls/htmldb_ pgrc/f?p=185:3:2422827336895397# (accessed, 12/11/14).

*/Marchand NL. 1869. Révision du groupe des Anacardiacées. Bailliére JB and Sons, Paris, France.

*/Martelli U. 1886. Florula Bogosensis. Enumerazione Delle Plante Dei Bogos. Firenze, Tipografia di Mariano Ricci. p. 24.

*/Mathey-Dupraz A.1924. Notes sur la flore du département d'Alger. *Bulletin de la Société Neuchâteloise des Sciences Naturelles.*

*/McNeil M, Darvill AG, Fry SC and Albersheim P. 1984. Structure and function of the primary cell walls of plants. *Annual Review of Biochemistry*. 53:625-663.

*/Mecherara-Idjeri S, Hassani A and Casanova J. 2008. Composition of leaf, fruit and gall essential oils of Algerian *Pistacia atlantica* Desf., *Journal of Essential Oil Research*, 20:215-219.

*/Medail F and Quezel P. 2003. Conséquences écologiques possibles des changements climatiques sur la flore et la végétation du bassin méditerranéen. *Bocconea*, 16(1): 000-000. 2003. - ISSN 1120-4060.

*/Meddi M and Hubert P. 2002. Impact de la modification du régime pluviométrique sur les ressources en eau du Nord-ouest de l'Algérie. Hydrology of die Mediterranean and Semiarid Regions (Proceedings of the International Symposium Held at Montpellier, April. IAHS Publications78: 229-235.

*/Meddi M, Talia A et Martin C. 2009. Évolution récente des conditions climatiques et des écoulements sur le bassin versant de la Macta, Nord-Ouest de l'Algérie. Physio-Géogo : Géographie Physique et Environnement, 3 : 61-84. doi : 10.4000/physio-geo.686.

*/Meddour R. 2010. Bioclimatologie, phytogeographie et Phytosociologie en Algérie. Exemple des groupements forestiers et Préforestiers de la kabylie djurdjureenne. Thès. doc Univ Tizi Ouzou. P54.

*/Mehdeb J. 2011. Etude de la variabilité du Pistachier de l'Atlas *Pistacia atlantica* Desf. dans la région de Tiaret. Mém. Magister Univ. Tiaret. Algérie. 72p.

*/Mehra PN. 1976. Cytology of Himalayan Hardwoods. Sree Saraswaty Press, Calcutta.

*/Mehra PN and Sareen ETS. 1969. In IOPB Chromosome Number Reports XXII. *Taxon*, 18, 433-442.

*/Meinzer FC, Goldstein GH, Rundel PW. 1985. Morphological changes along an altitudinal gradient and their consequences for an andeangiant rosette plant. *Oecologia* 65: 278–283.

*/Metcalfe CR and Chalk L. 1950. Anatomy of the dicotyledons. Clarendon Press, Oxford.

*/Meyer S, Cerovic ZG, Goulas Y, Montpied P, Demotes-Mainard S, Bidel LPR, Moya I and Dreyer E. 2006. Relationships between optically assessed polyphenols and chlorophyll contents, and leaf mass per area ratio in woody plants: a signature of the carbonnitrogen balance within leaves? *Plant, Cell and Environment*, 29:1338-1348.

*/Michielin E, Rosso S, Franceschi E, Borges G, Corazza M, Oliveira J and Ferreira S. 2009. High-pressure phase equilibrium data for systems with carbon dioxide, α-humulene and trans-caryophyllene, *Journal of Chemical Thermodynamics*, 41:130-137.

*/Mitchell JD and Mori SA. 1987. The cashew and its relatives (*Anacardium* : Anacardiaceae). Memoirs of the New York Botanical Garden 42: 1-76 .

*/Moisselin J, Schneider M, Canellas C and Mestre O. 2002. Les changements climatiques en France au xx siècle : Etudes des longues séries homogénéisées de données de température et de précipitations, *La Météorologie*, 38 : 45-56.

*/Monjauze A. 1968. Répartition et écologie de *Pistacia atlantica* Desf. En Algérie. *Bulletin de la Société Naturelle d'Afrique du Nord,* 56: 10-43.

*/Monjauze A. 1980. Connaissance du bétoum *Pistacia atlantica* Desf. *Biologie forestière*, 4 (32) : 356-363.

*/Monjauze A. 1982. Le pays des dayas et *Pistacia atlantica* Desf. dans le Sahara algérien. *Revue Forestière Française*, 34 : 277-289.

*/Mouri-Bendimered FZ. 2014. Etude des caractères morpho-physiologiques et biochimiques d'adaptation aux conditions naturelles et de stress d'une plante xérophile et psammophile, *Ammophila arenaria*(L.) Link. du littoral oranais. Thes. Doc. Univ DL SBA. 224 p + annexes.

*/Montanari RM, Barbosa LCA, Demuner AJ, Silva CJ, Andrade NJ, Ismail FMD and Barbosa MCA. 2012. Exposure to *Anacardiaceae* Volatile Oils and Their Constituents Induces Lipid Peroxidation within Food-Borne Bacteria Cells. *Molecules*, 17 : 9728-9740.

*/Mouton JA. 1967. La biométrie du limbe : mise au point de nos connaissances. *Bulletin de la Société botanique Française*, 123:145-158.

*/Muller J. 1984. Significance of fossil pollen for angiosperm history. Annals of the Missouri Botanical Garden, 71: 419-443.

*/Muller NJC. 1866-1867. Untersuchungen uber die Vertheilung der Harze, atherischen Oele, Gummi und Gummiharze, und die Stellung der Secretionsbehalter im Pflanzenkorper. Jahrbücher für Wissenschaftliche Botanik. Berlin.5: 387-439.

*/Mullis KB and Faloona FA. 1987. Specific synthesis of DNA in vitro via a polymerasecatalyzed chain reaction. *Methods in Enzymology*, 155:335-350.

*/Murchie EH and Horton P. 1997. Acclimation of photosynthesis to irradiance and spectral quality in British plant species: Chlorophyll content, photosynthetic capacity and habitat preference", *Plant, Cell and Environment*, 20:438-448.

*/Musset R. 1953. Les régimes pluviométriques saisonniers dans l'Ouest de la France et particulièrement en Normandie. *Bulletin de la Société Linnéenne de Normandie* 8 (7) : 49-65.

[N]

*/Nadkarni KM. 1908. Indian Materia Medica. Popular Prakashan PVT.LTD. Mumbai 2, p. 975.

*/Natarajan G. 1978. In IOPB Chromosome Number Reports LXII. *Taxon*, 27, 519-535.

*/Ndong M, Wade S, Dossou N, Guiro TA and Gning RD. 2007. Valeur nutritionnelle du Moringa oleifera, étude de la biodisponibilite du fer, effet de l'enrichissement de divers plats traditionnels sénégalais avec la poudre des feuilles. *African Journal of Food Agriculture Nutrition and Development*, 7(3): 1-17.

*/Nei M. 1973. Analysis of gene diversity in subdivided populations. Proceeding of National Academy of Sciences. USA 70: 3321-3323.

*/Nei M and Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceeding of National Academy of Sciences. USA 76: 5269-5273.

*/Nilsson Q and Lassen P. 1971. Chromosome Numbers of Vascular Plants from Austria, Mallorca and Yugoslavia. Botaniska Notiser, 124, 270-276.

*/Noge K and Becerra JX. 2009. Germacrene D, a common sesquiterpene in the genus *Bursera* (*Burseraceae*) *Molecules*, 14, 5289-5297; doi:10.3390/molecules14125289

[O]

*/Ohri D. 1998. Genome size variation and plant systematics. *Annals of Botany*, 82 (Suppl. A): 75-83.

*/Ohri D. 2005. Climate and growth form: the consequences for genome size in plants. *Plant Biology* 7: 449-458.

*/Ohtat T and Kimura M. 1973a. A new model for estimating the number of electrophoretically detectable alleles in a finite population. *Genetics*, 74: 201.

*/Ohtat T and Kimura M. 1973b. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetic Resources*, 22 (2):201-204.

*/ONM (Office National de Météorologie). 1981- 2011. Données Météorologiques des stations météorologiques de Maghnia, Safsaf, Ouled Mimou (Tlemcen) ; de Sidi Bel Abbes et de Mascara.

*/Ould Ahmed M, Ben Salem F, Bedhiaf S, Rekik B and Djemali M. 2010. Genetic diversity in Tunisian dromedary (***Camelus dromedarius***) populations using microsatellite markers. *Livestock Science* 132 (1-3): 182-185. 10.1016/j.livsci.2010.05.016

***/**Oxfam. 2006. Causing Hunger: An Overview of the Food Crisis in Africa. Briefing Paper. 39 pp. http://www.oxfam.org/en/policy/briefingpapers/bp91_africa_food_crisis.

*/Ozbek S and Ayfer M. 1957. *Pistacia* turleri uzerinde sitolojik arastirmalar. Ankara, Univ. Ziraat Fakultesi Yilligi, 3: 203-222.

*/Ozçimen D and Karaosmanoglu F. 2004. Production and characterization of bio-oil and biochar from rapeseed cake. *Renewable Energy*, 29, 779-787.

*/Ozenda P. 1983. Flore du Sahara.: CNR Ed. 622p.

[P]

*/Paetkau D 2003. An empirical exploration of data quality in DNA-based population inventories. *Molecular Ecology*, 12:1375-1387.

*/Paetkau D, Calvert W, Stirling I and Strobeck C. 1995. Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, 4:347-54.

*/Painting KA, Perry MC, Denning RA and Ayad WG. 1993. Guidebook for genetic resources documentation. IBPGR, Rome.

*/Parfitt DE and Badenes ML. 1997. Phylogeny of the genus Pistacia as determined from analysis of the chloroplast genome. Proceeding National Acaddemy of Sciences. USA 94, 7987-7992.

*/Parida SK, Yadava DK and Mohapatra T. 2010. Microsatellites in *Brassica unigenes*: relative abundance, marker design, and use in comparative physical mapping and genome analysis. *Genome*, 53:55-67.

*/Pazouki L, Mardi M, Shanjani PS, Hagidimitriou M, Pirseyedi SM, Naghavi MR, Avanzato D, Vendramin E, Kafkas S, Ghareyazie S, Ghaffari MR, Khayam and Nekoui MR. 2010. Genetic diversity and relationships among *Pistacia* species and cultivars. *Conservation Genetics*, 11:311-318; doi 10.1007/s10592-009-9812-5.

*/Pazouki L, Salehi Shanjani P, Hagidimitriou M, Pirseyedi SM, Naghavi MR, Avanzato D, Quarta R, Kafkas S, Ghareyazie B, Ghaffari MR, Khayam Nekoui SM, Mardi M. 2009. Genetic diversity and relationships among Pistacia species and cultivars. 5th International Symposium on Pistachios and Almonds. Abstract book, p. 81, October 6-10, Sanlıurfa, Turkey.

*/Pell SK. 2004. Molecular systematics of the cashew family (Anacardiaceae). PhD Dis. Louisiana State University, Baton Rouge, LA, USA.

*/Pellicer J, Fay MF, Gardens RB and Tw RS. 2010. The largest eukaryotic genome of them all ? 10-15.

*/Pons A and Quezel P. 1958. Premières remarques sur l'étude palynologique d'un guano fossile du Hoggar. Comptes Rendus de l'Académie des Sciences de Paris, 224: 2290-2292.

*/Poczai P, Varga I, Laos M, Cseh A, Bell N, Valkonen JPT and Hyvönen J. 2013. Advances in plant gene-targeted and functional markers: a review. *Plant Methods*, 9:6. http://www.plantmethods.com/content/9/1/6.

*/Powell W, Machray GC and Provan J. 1996. Polymorphism revealed by simple sequence repeats. *Trends in Plant Science*, 1 (7): 215-222, ISSN 1360-1385.

*/Preston RD. 1986. Natural cellulose. *In* Young RA and Rowell RM. (eds.), Cellulose: Structure, modification and hydrolysis, 3-27. New York: Wiley-Interscience.

*/Provan J, Powell W and Hollingsworth PM. 2001. Chloroplast microsatellites: new tools for studies in plant ecology and evolution. *Trends in Ecology and Evolution*, 16:142-147.

[Q]

*Qin S, Sun Y, Shi C, He L, Meng Y and Ren X. 2012. Deacidification of *Pistacia chinensis* Oil as a promising nonedible feedstock for biodiesel production in China. *Energies*, 5, 2759-2770.

*/Quereda Sala J, Gil Olcina A, Perez Cuevas A, Olcina Cantos J, Rico Amoros A and Monton Chiva E. 2000. Climatic warming in the spanish mediterranean : natural trendor urban effect, *Climate Change*, 46, 473-483.

*/Quezel P. 1960. Flore et palynologie saharienne. *Bulletin de l'Institut Fondamental d'Afrique Noire*, 22 (A2): 353-359.

*/Quezel P. 1985. Definition of the Mediterranean region and the origin of its flora (9-24). *In* C. Gomez-Campo (ed.), Plant Conservation in the Mediterranean Area. Junk, Dordrecht: 9-24.

*/Quezel P and Barbero M. 1985: Carte de la végétation potentielle de la région méditerranéenne. Feuille n° 1 : Méditerranée Orientale, Paris.

*/Quezel P, Santa S. 1963. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Centre National de la Recherche Scientifique, Paris T2, Pp : 611,612.

[R]

*/Rajendrakumar P, Biswal AK, Balachandran SM, Srinivasarao K and Sundaram RM. 2007. Simple sequence repeats in organellar genomes of rice: frequency and distribution in genic and intergenic regions. *Bioinformatics*, 23: 1-4.

*/Rajsnerová P, Klem K, Holub P, Novotná K, Vecerová K, Kozáciková M, Rivas-Ubach A, Sardans J, Marek MV, Peñuelas J and Urban O. 2015. Morphological, biochemical and physiological traits of upper and lower canopy leaves of European beech tend to converge with increasing altitude. Tree Physiology 35, 47-60 doi:10.1093/treephys/tpu104

*/Ramirez JL and Cevallos-Ferriz SRS. 2002. A diverse assemblage of Anacardiaceae from Oligocene sediments, Tepexi de Rodriguez, Puebla, Mexico. *American Journal of Botany*, 89,535-545.

*/Ranjbarfordoei A, Samson R, Van Damme P and Lemeur R. 2001. Effets of drought stress induced by polyethylene glycol on pigment content and photosynthetic gas exchange of *Pistacia khinjuk* and *P. mutica*. *Photosynthetica*, 38: 443-447, doi:10.1023/A:1010946209484.

*/Raven PH. 1975. The bases of angiosperm phylogeny: Cytology. *Annals of the Missouri Botanical Garden, The Bases of Angiosperm Phylogeny*, 62 (3): 724-764.

*/Raynal R. 1996. Procédé de fractionnement des sons de blé - Extraction et étude des propriétés fonctionnelles des arabinoxylanes., Thés., INP Toulouse.

*/Rechinger KH. 1963. Anacardiaceae. *In*: Rechinger KH (ed) Flora Iranica, Akademische Druck- und Verlagsanstalt, Graz, 63: 1-9.

*/Rechinger KH. 1969. Flora Des iranischen hochlandes und der umrahmenden gebirge, N°.63. Akademische Druck-u. Verlagsanstalt, Graz (Austria).

*/Reeve D. 2005. Perfum. *Flavor*, 30: 32-34.

*/Rendle AB. 1925. The classification of flowering plants. Cambridge University Press, Cambridge.

*/Rice EL. 1979. Allelopathy – *An update Botanic Review*, 45, 15-109.

*/Rol R. 1937. Études sur la répartition des essences forestières en France. Contribution à l'étude de la répartition du Sapin (Abies alba Mill.). *Annales de l'École Nationale des Eaux et Forêts et de la Station de Recherches et Expériences forestières*, 6, (2) : 1-68.

*/Roitman JN, Merrill GB and Beck JJ. 2011. Urvey of ex situ fruit and leaf volatiles from several *Pistacia* cultivars grown in California. *Journal of the Science of Food and Agriculture*, 91 (5): 934-942.

*/Rouskas D. 1996. Conservation strategies of *Pistacia* genetic resources in Greece. *In* Padulosi S, Caruso T, Barone E (eds). Taxonomy, distribution, conservation and uses of *Pistacia* genetic resources. Report of the IPGRI workshop 29-30 June 1995 Palermo, Italy. 37-41.

[S]

*/Sandhu PS and Mann SK. 1988. SOCGI Plant Chromosome Number Reports VII. *Journal of Cytology and Genetics*, 23, 219-228.

*/Savolainen V, Chase MW, Hoot SB, Morton CM, Soltis DE, Bayer C, Fay MF, De Bruijn AY, Sullivan S and Qiu YL. 2000a. Phylogenetics of flowering plants based on combined analysis of plastid atpB and rbcL gene sequences. *Systematic Biology*, 49:306-362.

*/Savolainen V, Fay MF, Albach DC, Backlund A, Van Der Bank M, Cameron KM, Johnson SA, Lledó MD, Pintaud J-C, Powell M, Sheahan MC, Soltis DE, Soltis PS, Weston P, Whitten WM, Wurdack KJ and Chase MW. 2000b. Phylogeny of the eudicots: a nearly complete familial analysis based on rbcl gene sequences. *Kew Bulletin*, 55:257-309.

*/Shapiro H. 2004. Practical flow cytometry. Wiley-Liss, New York.

*/Shapiro H. 2007. Cytometry and cytometers: development and growth. - In: Doležel J, Greilhuber J and Suda J (eds), Flow cytometry with plant cells, p. 1-17, Wiley-VCH, Weinheim.

*/Schär C, Vidale P, Lüthi D, Frei C, Häberli C, Liniger M, and Appenzeller C. 2004. The role of increasing temperature variability in european summer heatwaves, *Nature*, 427.

*/Schwarzacher T and Heslop-Harrison P. 2000. Practical *in situ* Hybridization. - BIOS Scientific Publishers,. Oxford . Paperback, 216 pp.

*/Selmar D and Kleinwächter M. 2013. Stress enhances the synthesis of secondary plant products: the impact of the stress-related over-reduction on the accumulation of natural products. *Plant and Cell Physiology*, 54(6):817-26.

*/Seltzer P. 1946. Le climat de l'Algérie. Alger, Carbonel. 219 p.

*/Serpico M. 1996. Mediterranean Resin Trade in New Kingdom Egypt, Unpublished Ph.D thesis, University College London.

*/Shure DJ and Wilson L A. 1993. Patch-size effects on plant phenolics in successional openings of the southern appalachians. *Ecology*, 74, 55-67.

*/Sieck W. 1895. Die schizolysigenen Secretbehalter Jabrb. Wiss. Bot. 27: 197-242.

*/Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior M L, Mitchell SE, Kresovich S and Zeigle J. 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparison with data from RFLPs and pedigree. *Theorical and Applied Genetics*, 95, 163-173.

*/Simson B W and Timell TE. 1978a. Polysaccharides in cambial tissues of *Populus tremuloides* and *Tilia americana*. I. Isolation, fractionation and chemical composition of the cambial tissues. *Cellulose Chemistry and Technology*, 12, 39-50.

*/Simson B W and Timell TE. 1978b. Polysaccharides in cambial tissues of *Populus tremuloides* and *Tilia americana*. IV. 4-O-methylglucuronoxylan and pectin. *Cellulose Chemistry and Technology*, 12, 79-84.
*/Skold M, Karlberg AT, Matura M and Borje A. 2006. The fragrance chemical β- Caryophyllene - air oxidation and skin sensitization, *Food and Chemical Toxicology*, 44: 538-545.

*/Soranzo N, Provan J and Powell W. 1999. An example of microsatellite length variation in the mitochondrial genome of conifers. *Genome*. 42: 158-161.

*/Sosa ME, Lancelle HG, Tonn CE, Andres MF and Gonzalez-Coloma A. 2012. Insecticidal and nematicidal essential oils from Argentinean *Eupatorium* and *Baccharis* spp. *Biochemical Systematics and Ecology*, 43 : 132–138 doi:10.1016/j.bse.2012.03.007.

*/Spach ME. 1834. Histoire naturelle des végétaux phanérogames. vol. 2, p11, Librairie Encyclopedique de Roret. Paris.

*/Stambouli-Essassi S. 2003 Etude palynologique en Tunisie septentrionale et reconstitution de l'évolution de l'environnement de 40 000 ans à l´époque présente. Thès. Univ. Liège Belgique.

*/Stenström A, Jónsdóttir I and Augner M. 2002. Genetic and environmental effects on morphology in clonal sedges in the eurasian arctic. *American Journal of Botany*, 89(9): 1410-1421.

*/Stewart P. 1969. Quotient pluviothermique et dégradation biosphérique. *Bulletin de la Société d'Histoire Naturelle d'Afrique du Nord,* 59: 23-36.

*/Stevens PF. 2008. Angiosperm Phylogeny Website, Version 9. http://www.mobot.org/MOBOT/research/Apweb/ (consulted februay 2014).

*/Stockmans F and Hooker. 1852. Notes on Beloochistan plants. Hooker's Journal of Botany and Kew Garden Miscellany 4: 143.

*/Stone C and Bacon PE. 1995. Leaf dynamics and insect herbivory in a *Eucalyptus camaldulensis* forest under moisture stress. *Australian Journal of Ecology*, 20, 473-481.

*/Stuessy TF. 1990. Plant taxonomy: The systematic evaluation of comparative data. New York: Columbia University Press.

*/Suda J, Kron P, Husband BC, Trávnícek P. 2007. Flow cytometry and ploidy: applications in plant systematics, Ecology and Evolutionary Biology. In: Dolezel J, Greilhuber J, Suda J, eds. Flow cytometry with plants cells. Weinheim: Wiley-VCH, 103-130.

*/Suc J-P. 1984. Origin and evolution of the Mediterranean vegetation and climate in Europe. *Nature*, 307 (5950): 429-432.

*/Suc J-P, Bertini A, Combourieu-Nebout N, Diniz F, Leroy S, Russo-Ermollm E, Zheng Z, Fauquette S and Ferrmr J. 1995. Structure of West Mediterranean and climate since 5,3 Ma. *Acta zoologica cracoviensis*, 38 (1): 3-16.

*/Svoboda K and Svoboda T. 2000. Secretory structure of aromatic and medicinal plants: a review and Atlas of micrographs, Microscopix Publication, Knighton.

*/Swift H. 1950. The constancy of desoxyribose nucleic acid in plant nuclei. Proceedings of the National Academy of Sciences of the USA 36: 643-654.

*/Swingle WT. 1920. A new species of *Pistacia* native to southwestern Texas, *P. texana*. *Journal of the Arnold Arboretum* 2: 105-110.

[T]

*/Takhtajan A. 1954. Origins of Angiospermous Plants. American Institute of Biological Sciences, Washington, D. C., USA.

*/Takhtajan A. 1997. Diversity and classification of flowering plants. Columbia University Press, New York, New York, USA.

*/Talebi M, Kazemi M and Sayed-Tabatabaei BE. 2012. Molecular diversity and phylogenetic relationships of *Pistacia vera*, *Pistacia atlantica* subsp. *mutica* and *Pistacia khinjuk* using SRAP markers. *Biochemical Systematics and Ecology*, 44 179-185.

*/Talhouk SN, Nehme GA, Baalbaki R, Zurayk R and Adham Y. 1998. Ecogeographic characterization of *Pistacia* spp. in Lebanon. *In*: Padulosi S, Hadj-Hassan A, (eds). 2001. Project on Underutilized Mediterranean Species. *Pistacia*: towards a comprehensive documentation of distribution and use of its

genetic diversity in Central and West Asia, North Africa and Mediterranean Europe. Report of the IPGRI Workshop, 14-17 December, Irbid, Jordan.

*/Tautz D. 1989. Hypervariability of simple sequences as a general source of polymorphic DNA markers. *Nucleic Acids Reseach*, 17:6463-6471.

*/Tautz D. 1993. Notes on the definition and nomenclature of tandemly repetitive DNA sequences, pp 21-28, *bz:* DNA Fingerprinting : State of the Science, Pena SDJ, Chakraborty R, Epplen JT and Jeffreys AJ (eds.). Birkhaiiser Verlag, Basel, Switzerland. ISBN 3-7643-2781-2.

*/Tautz D., Renz M., 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Research*, 12: 4127-4138.

*/Tavares AC, Loureiro J, Castro S, Coutinho AP, Paiva J, Cavaleiro C, Salgueiro L and Canhoto JM. 2014. Assessment of *Daucus carota* L. (Apiaceae) subspecies by chemotaxonomic and DNA content analyses *Biochemical Systematics and Ecology*, 55: 222-230.

*/Terrazas T. 1994. Wood anatomy of the Anacardiaceae: Ecological and phylogenetic interpretation. Ph.D. dissertation, university of North Carolina, Chapel Hill, North Carolina, USA.

*/Thorne RF. 1992. Classification and geography of the flowering plants. *Botanical Review*, 58(3): 225-348.

*/Throne RF and Reveal JL. 2007. An Updated Classification of the Class Magnoliopsida ("Angiospermae"). The Botanical Review, The New York Botanical Garden USA, 73(2): 67-182.

*/Tiffney BH. 1985. The Eocene North Atlantic land bridge: Its importance in Tertiary and modern phytogeography of the Northern Hemisphere. *Journal of Arnold Arboretum,* 66: 243-273.

*/Tiffney BH and Manchester SR. 2001. The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere Tertiary. *International Journal of Plant Science*, 162: 3-17.

*/Timell TE. 1965. Wood hemicellulose: Part ll. *Advances in Carbohydrate Chemistry*, 20, 409-483.

*/Tirse M, Benhassaini H, Sail K and Bassou Gh. 2014. Leaflets Epidermal Micro-Characters of *Pistacia atlantica* Desf. subsp. *atlantica* (Anacardiaceae) under Semi-Arid Environmental Factors. *Environmental research Journal,* 7 (5): 433-447.

*/Tissouras F. 2001. Contribution à l'étude de tournesol (*Helianthus annuus* L.) Germination, croissance, histologie et biochimie au cours du développement. U.S.T.O. Oran. (p.77).

*/Tournefort JP. 1700. Institutiones rei herbariae. Typographia Regia, Paris, France.

*/Trevor PD, Anna Q and Darren IE. 2003. The role of phenotypic plasticity in driving genetic evolution. Proceedings of the Royal Society B: *Biological Sciences* 270 (1523): 1433-40. doi:10.1098/rspb.2003.2372. PMC 1691402. PMID 12965006.

*/Tschirch A. 1900. Die Harze u.d. Harzbehalter. Gebruder Borntraeger, Leipzig.

*/Tsukaya H. 2003. Organ shape and size: a lesson from studies of leaf morphogenesis. *Current Opinion in Plant Biology*, 6: 57-62.

*Turtola S, Manninen A-M, Rikala R, Kainulainen P. 2003. Drought stress alters the concentration of wood terpenoids in Scots pine and Norway spruce seedlings. *Journal of Chemical Ecology*, 29:1981-1995.

*Tzakou O, Bazos I and Yannitsaros A. 2007. Volatile metabolites of *Pistacia atlantica* Desf. from Greece, *Flavour and Fragrance Journal*, 22 (5): 358-362.

[U]

*/Ulbrich U, May W, Lionello P, Pinto J and Somot S. 2005. Mediterranean Climate variability, chap. Chapter 8: The Mediterranean Climate Change Under Global Warming, Elsevier B.V.

*/UNEP-GEF RAB/94/G31 project, 2002. 6th Symposium of the North African Steering Committee on Climatic Changes: Vulnerability and Adaptation of North African Countries to Climate Change, Algiers, May 6-7.

[V]

*/Van Campo M. 1975. Pollen analysis in the Sahara. *In*: Wendorf F and Marks AE (eds), Problems in Prehistory: North Africa and the Levant, 45-64. Dallas TX: Southern Methodist University Press.

*/Van Zinderen Bakker B and Maley J. 1979. Late Quaternary palae-oenvironments of the Saharan region. *Palaeoecology of Africa*, 10(11): 83-104.

*/Vendramin E, Dettori MT, Verde I, Micali S, Giovinazzi J, Mardi M, Avanzato D and Quarta R. 2009.). Molecular characterization of *Pistacia* genus by microsatellite markers. *Acta Horticultura*.825,55-62, doi:10.17660/Acta Horticultura.2009.825.5.

*/Vendramin E, Gallego JA, Micali S, Giovinazzi J, Verde I, Dettori MT and Quarta R. 2010. Development and characterisation of fourteen EST-SSRs from *Pistacia vera* L. inflorescence transcriptome. *Molecular Ecology Resources*, 10 (3): 576-579

*/Vogt R and Aparicio A. 1999. Chromosome numbers of plants collected during Iter Mediterraneum IV in Cyprus. *Bocconea* 11: 117-169.

[W]

*/Wagner HW and Sefc KM. 1999. IDENTITY 1.0. Vienna, Centre for Applied Genetics, University of Agricultural Sciences.

*/Wannan BS and Quinn CJ. 1991. Floral structure and evolution in the Anacardiaceae. *Botanical Journal of the Linnean Society*, 107:349-385.

*/Webmaster1: http://www.palestineremembered.com/Safad/al-Nabi-Yusha'/ar/Picture7921.html (accessed on 10/11/2013)

*/Weeks A, Daly DC and Simpson BB. 2005. The phylogenetic history and biogeography of the frankincense and myrrh family (Burseraceae) based on nuclear and chloroplast sequence data. *Molecular and Phylogenetic Evolution*, 35, 85-101.

*/Welsh J and McClelland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research*, 18:7213-7218.

*/West-Eberhard M. 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology, Evolution, and Systematics*, 20:249-278.

*/Wettstein R, Hirmer M, Süssenguth K and Wettstein F. 1935. Handbuch der systematischen botanik. 1962 reprint by Asher & Company, Amsterdam, The Netherlands.

*/Wettstein R, Hirmer M, Süssenguth K and Wettstein F. 1944. Tratado de botánica sistemática. Editorial Labor SA, Barcelona, Spain.

*/Weyland H. 1941. Beiträge zur Kenntnis der Rheiniscehin Tertiarflora: V. Dritte Erganzungen und Berichtigungen zur flora der Blätterkohle und des Polierschiefers von Rott im Siebengebirge. *Paleontographic*, 86, 79-112.

*/Wikstrom N, Savolainen V and Chase MW. 2001. Evolution of the angiosperms: Calibrating the family tree. Proceeding of Biological Sciences, 268: 2211-2220.

*/Willis CG, Ruhfel B, Primack RB, Miller-Rushing AJ and Davis CC. 2008. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. Proceeding of the National Academy of Sciences. USA 105, 17029-17033.

*/Willis CG, Ruhfel B, Primack RB, Miller-Rushing AJ and Davis CC. 2010. Favorable climate change response explains non-native species' success in Thoreau's Woods. *PLoS* ONE 5 (1), e8878. doi: 10.1371/journal. pone. 0008878.

*/Wilson D and Cooper JP. 1969a. Effect of light intensity and CO2 on apparent photosynthesis and its relationship with leaf anatomy in genotypes of *Lolium perenne* L. *New Phytology*, 68, 627

*/Wright S. 1931. Evolution in Mendelian populations. *Genetics*, 16:97-159.

*/Wright S. 1951. The genetical structure of populations. *Annals of Eugenics*, 15: 323-354.

*/Wright S. 1978. Evolution and the genetics of population, variability within and among natural populations. The University of Chicago Press, Chicago.

*/Wolfe JA. 1978. A paleobotanical interpretation of Tertiary climates in the Northern Hemisphere. Amer5can Scientists, 66: 694-703.

*/Wolfe JA. 1995. Paleoclimatic estimates from tertiary leaf assemblages. *Annual Review of Earth and Planetary Sciences*, 23, 119-142.

[X]

*/Xoplaki E, Luterbacher J, Burkard R, Patrikas I and Maheras P. 2000. Connection between the large-scale 500 hpa geopotentiel height fields and precipitation over Greece during wintertime, Climate Research, 14, 129-146.

*/Xu F, Guo W, Xu W and Wang R. 2008. Habitat effects on leaf morphological plasticity in *Quercus acutissima*. *Acta Biologica Cracoviensia* Series *Botanica*, 50 (2): 19-26.

[Y]

*/Yaaqobi A, El Hafid L and Haloui B. 2009. Etude biologique de *Pistacia atlantica* Desf. de la région orientale du Maroc . Biomatec Echo, 6 (3) : 39-49.

*/Yaltirik F. 1967a. *Anacardiaceae*. *In*: Davis PH (ed) Flora of Turkey, Edinburgh University Press,2: 544-548

*/Yaltirik F. 1967 b. *Anacardiaceae*. Contributions to the taxonomy of woody plants in Turkey. Notes R Bot Gard Edinburgh, 28:11-12

*/Yang Y, Wang G, Klanderud K and Yang L. 2011. Responses in leaf functional traits and resource allocation of a dominant alpine sedge (*Kobresia pygmaea*) to climate warming in the Qinghai-Tibetan Plateau permafrost region. *Plant Soil* 349:377-387 doi 10.1007/s11104-011-0891-y.

*/Yeh FC, Yang RC, Boyle TJB, Ye ZH, Mao JX 1997. Popgene, the user-friendly shareware for population genetic analysis. *Molecular Biology and Biotechnology*. Center, Univ. of Alberta, Canada.

*/Yi T, Miller AJ and Wen J. 2004 . Phylogenetic and biogeographic diversifi cation of *Rhus* (Anacardiaceae) in the northern hemisphere. *Molecular Phylogenetics and Evolution,* 33: 861-879.

*/Yi T, Miller AJ and Wen J. 2007. Phylogeny of *Rhus* (Anacardiaceae) based on sequences of nuclear *NIA-i3* intron and chloroplast *trnCtrnD. Systematic Botany*, 32: 379-391.

*/Yi T, Wen J, Golan-Goldhirsh A and Parfitt DE. 2008. Phylogenetics and Reticulate Evolution in *Pistacia* (Anacardiaceae). *American Journal of Botany*, 95 (2) 241-251.

[Z]

*/Zachos J, Pagani M, Sloan L, Thomas E and Billups K. 2001. Trends, rhythms, and aberrations in global climate 65Ma to present. *Science*, 292: 686-693.

*/Zane L, Bargelloni L and Patarnello T. 2002. Strategies for microsatellite isolation: a review. *Molecular Ecology*, 11:1-16.

*/Zeitoun R. 2011. Procédés de fractionnement de la matière végétale. Application la production des polysaccharides du son et de la paille de blé. Thès de Doct Univ. de Toulouse (p. 291).

*/Zohary D. 1996. The genus *Pistacia* L. *In*: Padulosi S, Caruso T, Barone E (eds). Taxonomy, distribution, conservation and uses of *Pistacia* genetic resources. Report of the IPGRI workshop 29-30 June 1995 Palermo, Italy.

*/Zohary D. 2000. The taxonomic ranking of *Pistacia terebinthus* and *P. palaestinia*. FAO-CIHEAM-*Nucis-Newsletter*, 09: 39-40.

*/Zohary M. 1952. A monographical study of the genus *Pistacia*. Palestine. *Journal of Botany*, Jerusalem Series, 5: 187-228.

*/Zohary M. 1972. *Pistacia* L. Flora Palestine. Academy of Science and Humanities, Jerusalem 2:297-300.

*/Zonneveld BJM. 2010. New record holders for maximum genome size in eudicots and monocots. *Journal of Botany*. doi:10.1155/2010/527357.

APPENDIX

Natural Product Research, 2013
http://dx.doi.org/10.1080/14786419.2012.755679

Taylor & Francis
Taylor & Francis Group

Cellulosic and hemicellulosic fractions dosage of *Pistacia atlantica* Desf. subsp. *atlantica* leaves in western Algeria

El Zerey-Belaskri, A.* Benhassaini, H., Naimi, W. and Rahoui S.

Laboratoire de Biodiversité Végétale: Conservation et Valorisation, Faculté des Sciences de la nature et de la vie, Université de Sidi Bel Abbes, Algerie

(*Received 31 July 2012; final version received 27 November 2012*)

The aim of this study was to valorise a deciduous tree called *Pistacia atlantica*. Cell wall polysaccharides (cellulose and hemicelluloses) were isolated and dosed from mature and young pistachio leaves. The samples were collected from six different locations in the Sidi Bel Abbes region (western Algeria). The wall residue yield varied from 39.06% to 69.5%. The cellulose and the hemicellulose's cell wall yield varied from 21.25% to 40.6% and 0.11% to 13.6%, respectively. In the mature leaves, the cellulosic fraction is more important than the hemicellulosic fraction. The highest yield is noted in the young leaves.

Keywords: *Pistacia atlantica* Desf; cell wall polysaccharides; cellulose; hemicelluloses; valorisation; Sidi Bel Abbes

1. Introduction

Cell walls are essential to many fundamental processes in plants, such as growth and resistance. Most plant cells produce a primary wall which is much thinner than cytoplasmic membrane. Certain cells deposit additional layers inside the primary wall to form a secondary wall. Polysaccharides constitute a major proportion of the plant cell wall, depending, of course, on the species and the tissue under consideration. Cell walls define mainly the structure of plants. They play a role in plant defence against pathogens. Although all cell walls consist of celluloses, hemicelluloses and pectin polysaccharides, the proportions of each vary from species to species and with cell type. The variability can be observed also between populations of the same species. Cellulose occurs as crystalline, fibrillar aggregate of b-1,4-linked glucan chains (Frey-Wyssling, 1969). Different types of sugars constitute the hemicellulosic fraction in plant cell walls. The most common are xylans, arabinoxylans, galactomannans and xyloglucans (Timell, 1965).

Those polysaccharides have great practical significance including important role in the materials industry and in human and animal health as dietary fibre (Belhamri, 2005; Zeitoun, 2011). In addition, various lignocellulosic crops, non-recoverable for human food, are tested for the production of renewable energy vectors. The lignocellulosic biomass has been recognised as a major world renewable energy source to supplement declining fossil fuel resources (Ozçimen

*Corresponding author. Email: asma.belaskri@gmail.com

Among the multiple virtues of *P. atlantica*, its dense foliage appears to promise more interests in cell wall polysaccharides fields. According to the obtained results, the cellulosic fraction that varies from 21.25% to 40.6%, advantages the use of *P. atlantica* in the paper industry or as raw material for biofuels production.

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1 and S2.

References

Belhamri, R. (2005). *Extraction des macromolécules priétales des eaux de presse de betteraves cucrières. Etude de leur composition, de leurs propriétés physicochimiques et de leur effet sur le process sucrier* (Thèse de Doctorat). Université de Reims Champagne. Ardenne (p. 245).

Belyea, R.L., & Ricketts, R.E. (1993). *Forages for cattle: New methods of determining energy content and evaluating heat damage*. Columbia. MO: Univ. Missouri, Agricultural Publication G3150.

Bendimered, F.Z., & Mehdadi, Z. (data not published). Contribution à une étude des composants pariétaux (Polysaccharides) des parties aériennes et souterraines de l'oyat (*A. arenaria* L.).

Benhassaini, H., Benabderrahmane, M., & Chikhi, K. (2003). Contribution à l'évolution de l'activité antiseptique de l'oléorésine et des huiles essentielles du pistachier de l'Atlas sur certains sources microbiennes: *Candida albicans* (ATCC 20027), *Candida albican* (ATCC 20032) et *Saccbaromyces cerevisiae*. *Ethnopharamcologia*, *30*, 38–46.

Benhassaini, H., Bendahmane, M., & Benchalgo, N. (2007). The chemical composition of fruits of *Pistacia atlantica* Desf. subsp. *atlantica* from Algeria. *Chemistry of Natural Compounds*, *43*, 121–124.

Benhassaini, H., Bendeddouche, F.Z., Mehdadi, Z., & Romane, A. (2008). GC/MS analysis of the essential oil from the oleoresin of *Pistacia atlantica* Desf. subsp. *atlantica* from Algeria. *Natural Product Communication*, *3*, 929–932.

Bergmans, M.E.F., Beldman, G., Gruppen, H., & Voragen, A.G.J. (1996). Optimisation of the selective extraction of glucurono-arabinoxylans from wheat bran: Use of Barium and Calcium hydroxide solutions at elevated temperatures. *Journal of Cereal Science*, *23*, 235–245.

Bertrand, I., Chabbert, B., Kurek, B., & Recous, S. (2006). Can the biochemical features and histology of wheat residues explain their decomposition in soil? *Plant and Soil*, *281*, 291–307.

Bertrand, I., Prévot, M., & Chabbert, B. (2009). Soil decomposition of wheat internodes of different maturity stages: Relative impact of the soluble and structural fractions. *Bioresource Technology*, *100*, 155–163. doi: 10.1016/j.biortech.

Bessam, F.Z. (2008). *Contribution à l'étude de Stipa tenassicima L.: Germination Caractérisation de certains polyphénoles et effet d'obtention du caryotype* (96 p.). Algérie: Mem. Magis. Biodiv. Végét. Université Sidi Bel Abbes.

Bouzidi, M.A., Latrèche, A., Attaoui, I., Mehdadi, Z., Benyahia, M., Bouguenaya, N., & Meliani, H. (2010). Caractérisation et valorisation des polysaccharides pariétaux d'*Urginea pancration* (Steinh) Phil. de Djebel Tessala (Nord-Ouest Algérien). *Les Technol. de Labo.*, *5*(19), 23–29.

Carpita, N., & McCann, M. (2000). The cell wall. In B. Buchanan, W. Gruissem, & R. Jones (Eds.), *Biochemistry and molecular biology of plants* (pp. 52–108). Rockville, IN: American Society of Plant Physiologists.

Chenost, M., & Kayouli, C. (1997). Utilisation des fourrages grossiers en régions chaudes. *Etude FAO Production et santé Animales* (p. 135). Rome: Ed FAO.

Cindy, L., Wedig, E., Jaster, H., & Kenneth, J. (1987). Hemicelluloses monosaccharide composition and *in vitro* disappearance of orchard gross and Alfalfa hay. *Journal of Agricultural and Food Chemistry*, *35*, 214–218.

Cleland, R.E. (1977). The control of cell enlargement. *Symposia of the Society for Experimental Biology*, *31*, 101–115.

Demiral, I., Atilgan, N.G., & Sensoz, S. (2009). Production of biofuel from soft shell of pistachio (*Pistacia vera* L.). *Chemical Engineering Communications*, *196*, 104–115.

El Bouhissi, M. (2011). *Etude de* Asphodelus microcarpus *Salzm. et Viv. du Mont de Tessala (Algérie Occidentale): Histologie, Autoécologie et Dosage des Polysaccharides pariétaux* (p. 134). Algérie: Mem. Magis. Biol. Université Sidi Bel Abbes.

El Hamrouni, A., & Sarson, M. (1974). Valeur alimentaire de certaines plantes spontanées ou introduites en Tunisie. *Inst. Nat.de Rech. Fores, Notes de recherchenum*. vol. 2, 12 pp.

Frey-Wyssling, A. (1969). The ultrastructure and biogenesis of native cellulose. *Fortschritte der Chemie Organischer Naturstoffe*, *27*, 1–30.

Godin, B., Ghysel, F., Agneessens, R., Schmit, T., Gofflot, S., Lamaudière, S., … Delcarte, J. (2010). Détermination de la cellulose, des hémicelluloses, de la lignine et des cendres dans diverses cultures lignocellulosiques dédiées à la production de bioéthanol de deuxième génération (14) 2, numero spécial *Base* Gembloux. Belgique.

Houghton, J., Weatherwax, S., & Ferrell, J. (2006). Breaking the biological barriers to cellulosic ethanol: A joint research agenda. In J. Houghton, S. Weatherwax, & J. Ferrell (Eds.), *A research roadmap resulting from the biomass to biofuels workshop*, 7–9 December 2005, Rockville, MA: US Department of Energy.

Jeffries, T.W. (1994). *'Biodegradation of lignin and hemicelluloses.' Section in biochemistry of microbial degradation* C. Ratledge (Ed.), (pp. 233–277). Dordrecht: Kluwer Academic Publishers.

Ndong, M., Wade, S., Dossou, N., Guiro, T.A., & Gning, R.D. (2007). Valeur nutritionnelle du *Moringa oleifera*, étude de la biodisponibilite du fer, effet de l'enrichissement de divers plats traditionnels sénégalais avec la poudre des feuilles. *African Journal of Food Agriculture Nutrition And Development*, *7*(3), 1–17.

Ozçimen, D., & Karaosmanoglu, F. (2004). Production and characterization of bio-oil and biochar from rapeseed cake. *Renewable Energy*, *29*, 779–787.

Preston, R.D. (1986). Natural cellulose. In R.A. Young & R.M. Rowell (Eds.), *Cellulose: Structure, modification and hydrolysis* (pp. 3–27). New York: Wiley-Interscience.

Qin, S., Sun, Y., Shi, C., He, L., Meng, Y., & Ren, X. (2012). Deacidification of Pistacia chinensis Oil as a promising non-edible feedstock for biodiesel production in China. *Energies*, *5*, 2759–2770.

Quezel, P., & Santa, S. (1963). *Nouvelle flore de l'Algérie et des régions désertiques méridionales. Tome II* (pp. 611–612). Paris: Centre National de la Recherche Scientifique.

Raynal, R. (1996). Procédé de fractionnement des sons de blé – Extraction et étude des propriétés fonctionnelles des arabinoxylanes., Thèse, INP Toulouse.

Shiraishi, N., Tanahashi, M., Yokota T., & Yamada T. (1980). Thermal softening of wood and cellulose. *Wood Research and Technical Notes*, Tokyo, *15*, 61–72.

Simson, B.W., & Timell, T.E. (1978a). Polysaccharides in cambial tissues of *Populus tremuloides* and *Tilia americana.* I. Isolation, fractionation and chemical composition of the cambial tissues. *Cellulose Chemistry and Technology*, *12*, 39–50.

Simson, B.W., & Timell, T.E. (1978b). Polysaccharides in cambial tissues of *Populus tremuloides* and *Tilia americana.* IV. 4-O-methylglucuronoxylan and pectin. *Cellulose Chemistry and Technology*, *12*, 79–84.

Timell, T.E. (1965). Wood hemicellulose: Part ll. *Advances in Carbohydrate Chemistry*, *20*, 409–483.

Tissouras, F. (2001) Contribution à l'étude de tournesol (*Helianthus annuus* L.) Germination, croissance, histologie et biochimie au cours du développement. U.S.T.O. Oran. (p.77).

Zeitoun, R. (2011). *Procédés de fractionnement de la matière végétale. Application la production des polysaccharides du son et de la paille de blé* (Thèse de Doctorat). Université de Toulouse (p. 291).

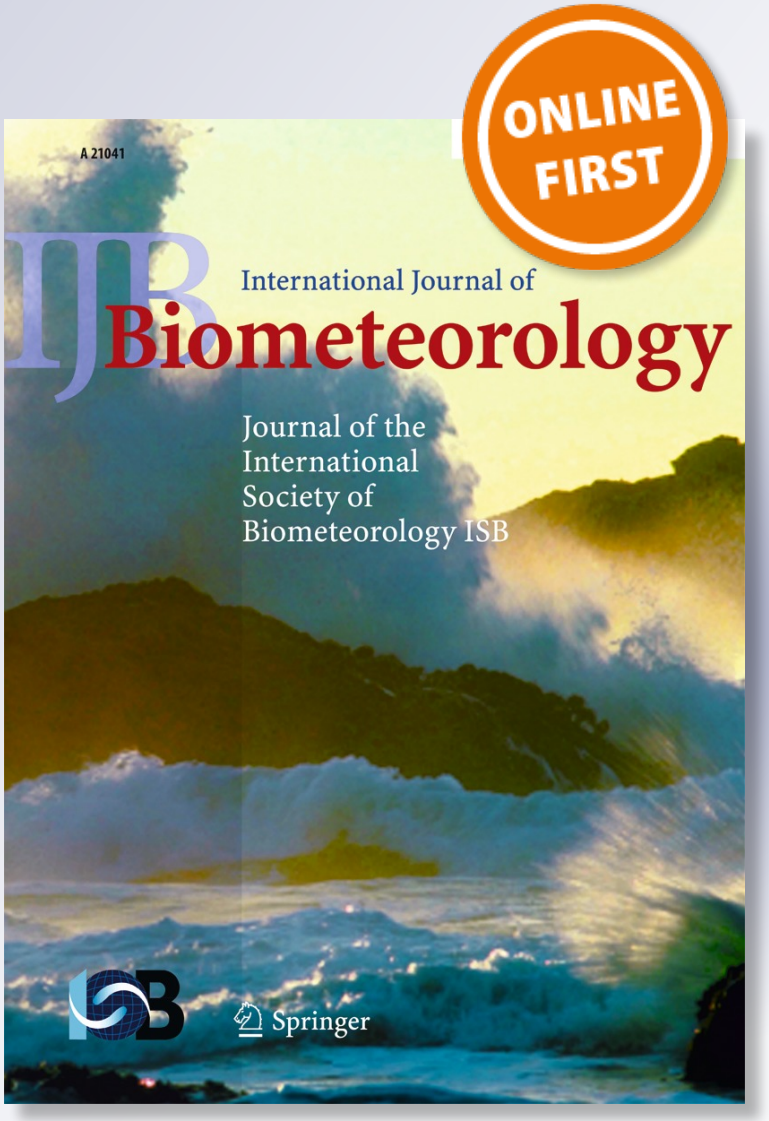
Morphological leaf variability in natural populations of Pistacia atlantica *Desf. subsp.* atlantica *along climatic gradient: new features to update* Pistacia atlantica *subsp.* atlantica *key*

Asma El Zerey-Belaskri & Hachemi Benhassaini

International Journal of Biometeorology

ISSN 0020-7128

Int J Biometeorol
DOI 10.1007/s00484-015-1052-4



Springer

Int J Biometeorol
DOI 10.1007/s00484-015-1052-4

ORIGINAL PAPER

Morphological leaf variability in natural populations of *Pistacia atlantica* Desf. subsp. *atlantica* along climatic gradient: new features to update *Pistacia atlantica* subsp. *atlantica* key

Asma El Zerey-Belaskri[1,2] · **Hachemi Benhassaini**[1,2]

Received: 31 October 2014 / Revised: 27 July 2015 / Accepted: 14 August 2015

Abstract The effect of bioclimate range on the variation in *Pistacia atlantica* Desf. subsp. *atlantica* leaf morphology was studied on 16 sites in Northwest Algeria. The study examined biometrically mature leaves totaling 3520 compound leaves. Fifteen characters (10 quantitative and 5 qualitative) were assessed on each leaf. For each quantitative character, the nested analysis of variance (ANOVA) was used to examine relative magnitude of variation at each level of the nested hierarchy. The correlation between the climatic parameters and the leaf morphology was examined. The statistical analysis applied on the quantitative leaf characters showed highly significant variation at the within-site level and between-site variation. The correlation coefficient (r) showed also an important correlation between climatic parameters and leaf morphology. The results of this study exhibited several values reported for the first time on the species, such as the length and the width of the leaf (reaching up to 24.5 cm/21.9 cm), the number of leaflets (up to 18 leaflets/leaf), and the petiole length of the terminal leaflet (reaching up to 3.4 cm). The original findings of this study are used to update the *P. atlantica* subsp. *atlantica* identification key.

Keywords Climatic variation · Morphological leaf variability · *Pistacia atlantica* Desf. subsp. *atlantica* · Identification key

Introduction

Many studies are focused on the impact of climatic changes on the genetic variability and the long-term survival of living beings. This issue is the most important since the current fragmentation of natural habitats strongly restricts the opportunities of the dispersal of species as well as the gene flow among populations. When a species exhibits a large range, the fragmentation of the habitats and the adaptive potentialities to the ecological variability may lead to intraspecific subdivisions, breeding subspecies, varieties, and ecotypes. This is the case of the Atlas pistachio, *Pistacia atlantica* Desf. It is an Irano-Touranian species with a large geographic range (Zohary 1952). It would have existed in the Mediterranean region since the dry and the cold climatic phases of the Miocene. The Plio-Pleistocene glaciations were propitious for pistachio establishment in the region. Although its range is more or less disjunct, *P. atlantica* is one of the most widely distributed wild species of the genus. It occurs from the Canary Islands to the Pamir mountains (Zohary 1952).

Over its large distribution area and under different ecological conditions, *P. atlantica* populations adapt differently; the morphological characters of the species are very variable, leading to taxonomic confusion. Many revisions in the species level (between subspecies) were proposed. The last subspecies description was done by Al Yafi (1978, 1979) who described four subspecies for *P. atlantica*. He retained Rechinger's subdivision adding *P. atlantica* subsp. *atlantica* which represents the species in North Africa. This classification was based on morphological characters, while Kafkas and Perl-Treves

Electronic supplementary material The online version of this article (doi:10.1007/s00484-015-1052-4) contains supplementary material, which is available to authorized users.

✉ Asma El Zerey-Belaskri
asma.belaskri@gmail.com

[1] Laboratoire de recherche 'Biodiversité Végétale: Conservation et Valorisation' Faculté des Sciences de la Nature et de la vie, Université de Sidi Bel Abbes, Sidi Bel Abbes, Algérie

[2] Département des Sciences de l'Environnement, Faculté des Sciences de la Nature et de la Vie, bp 89. 22000 SBA, Sidi Bel Abbes, Algérie

Published online: 02 November 2015

characterization is recommended to study the genetic intraspecific relationships in *P. atlantica* subsp. *atlantica*.

Acknowledgments The authors are grateful to the anonymous reviewers for their pertinent comments. Special acknowledgments go to the different meteorological stations of the "National Office of Meteorology" for providing the climatic data and to Mr. Youcef Khelifi (Ingénieur d'Etat en Biologie, univ. Sidi Bel Abbes) for his efficient help in the treatment of the climatic data.

Competing interests The authors declare that they have no competing interests.

References

Aidoud A (1989) Les écosystèmes steppiques pâturés d'Algérie: fonctionnement, évaluation et dynamique des ressources végétales. Thèse Doct Univ Sci Technol H. Boumédiene Alger 250 p. + ann.

Ait Said S, Fernandez C, Greff S, Derridj A, Gauquelin T, Mevy JP (2011) Inter-population variability of leaf morphoanatomical and terpenoid patterns of *Pistacia atlantica* Desf. ssp. *atlantica* growing along an aridity gradient in Algeria. Flora 206(4):397–405

Al Yafi J (1978) New characters differentiating *Pistacia atlantica* subspecies. Candollea 33:201–208

Al Yafi J (1979) Approches systématique et écologique du genre *Pistacia* L. dans la région méditerranéenne. Thése doc Univ. D'Aix-Marseille St Jérome France.

Alcaraz C (1969) Principaux types de successions géographiques concernant le pin d'alep et ses principales espèces-climax concurrentes dans le Tell oranais. Bull Soc Hist Mat fr Nord 60: 103–115

Al Saghir MG, Porter DM (2012) Taxonomic revision of the genus *Pistacia* L. (Anacardiaceae). Am J Plant Science 3:12–32

Angot A (1981) Étude sur le climat de l'Algérie (température, pression barométrique et pluie). Ann Bur Cent Météo Paris 13:7–36

Behboodi BS (2005) Ecological distribution study of wild pistachios for selection of roostock. In: Oliveira MM (ed.), Cordeiro V. (ed.). XIII GREMPA Meeting on Almonds and Pistachios. Zaragoza: CIHEAM, Option Méd 63: 61–67.

Belhadj S, Derridj A, Auda Y, Gers C, Gauquelin T (2008) Analyse de la variabilité morphologique chez huit populations spontanées de *Pistacia atlantica* en Algérie. Botany 86:520–532

Benhassaini H, Mehdadi Z, Hamel L, Belkhodja M (2007) Phytoécologie de *Pistacia atlantica* Desf. subsp. *atlantica* dans le Nord-Ouest algérien. Sécheresse 18(3):199–205

Besancenot JP (1986) Bioclimatologie méditerranéenne. Ann Géog 95(532):709–715

Boudy P (1952) Guide forestier en Afrique du Nord. (ed)La Maison Rustique, Paris, p196.

Charnay L (2001) Précision de l'écologie des essences forestières dans les Alpes à l'aide de données spatialisées. Nancy : ENGREF, 55 p. (Mémoire de 3e année FIF).

Chenoune K (2005) la flore et la végétation du Hoggar. Bois For Trop 284(2):79–83

Daget P (1977) Le bioclimat méditerranéen : caractères généraux, modes de caractérisation ? Végétation 34:1–20

De Martonne E (1926) Aréisme et indice d'aridité. Compt Rend Acad Science 181:1395–1398

Debrach J (1953) Notes sur les climats du Maroc occidental. Maroc Oriental 32(242):1122–1134

Desfontaines RL (1799) *Flora atlantica*; *sive historia platarium quae in atlante*, *agro Tunetano et Algeriensi crescent*. Paris, L G Desgranges, 2: 364–364.

Douglas DA (1981) The balance between vegetative and sexual reproduction of *Mimulus primuloides* (Scropholariaceae) at different altitudes in California. J Ecol 69:295–310

Emberger L (1941) Les limites de l'aire de végétation méditerranéenne en France. Bull Soc Hist Nat Toulouse, 1943, 78, 3, 159–180.

Emberger L (1942) Un projet de classification des climats du point de vue phytogéographique. Bull Soc Hist Nat Toulouse 77:97–124

Emberger L (1955) Une classification biogéographique des climats. Trav Lab Bot Zool Fac Sci Serv Bot Montpellier 7:3–43

Engler A (1883) *Anacardiaceae*. In: De Candolle, A. L. P. (ed.), Monographiae phanerogamarum.

Fahn A, Cutler DF (1992) Xerophytes. Gebrüder Borntraeger, Berlin, p. 180

Ghorbel A, Ben Salem-Fnayou A, Chatibi A, Twey M (1998) Genetic resources of *Pistacia* in Tunisia. *In* Padulosi S, Hadj Hassan A (eds.): Towards a comprehensive documentation and use of *Pistacia* genetic diversity in Central and West Asia, North Africa and Europe. Report of the IPGRI Workshop, 14–17 December: 62–71.

IPGRI (1998) Descriptors for *Pistacia* spp (excluding *P. vera* L.). International Plant Genetic Resources Institute, Rome.

Kadi-Bennane S, Ait-Said S, Smail-Saadoun N (2005) Etude adaptative de trois populations de *Pistacia atlantica* Desf. ssp. *atlantica* (Ain Oussera - Messaad - Taissa) par le biais du complexe stomatique. Options Méd 63:365–368

Kafkas S, Perl-Treves R (2001) Morphological and molecular phylogeny of *Pistacia* species in Turkey. Theor Appl Gen 102:908–915

Kafkas S, Perl-Treves R (2002) Interspecific relationships in *Pistacia* based on RAPD fingerprinting. Hort Science 37:168–171

Kafkas S, Kafkas E, Perl-Treves R (2002) Morphological diversity and a germplasm survey of three wild *Pistacia* species in Turkey. Genet Res Crop Evol (49) 261: 261–270

Karimi HR, Zamani Z, Ebadi A, Fatahi MR (2009) Morphological diversity of *Pistacia* species in Iran. Genet Resour Crop Evol 56:561–571

Körner C, Neumayer M, Menendez-Riedl SP, Smeetsscheel A (1989) Functional morphology of mountain plants. Flora 182:353–383

Ludlow (1983) In: Dale JE, Milthorpe FL (eds) The growth and functioning of leaves. Cambridge University Press, Cambridge, pp. 347–380

Medail F, Quezel P (2003) Conséquences écologiques possibles des changements climatiques sur la flore et la végétation du bassin méditerranéen. Bocconea 16(1):000–000 ISSN 1120-4060

Meddi M, Hubert P (2002) Impact de la modification du régime pluviométrique sur les ressources en eau du Nord-Ouest de l'Algérie. Hydrology of die Mediterranean and Semiarid Regions (Proceedings of an international symposium held at Montpellier, April. IAHS Publ 78:229–235

Meinzer FC, Goldstein GH, Rundel PW (1985) Morphological changes along an altitudinal gradient and their consequences for an andeangiant rosette plant. Oecologia 65:278–283

Monjauze A (1968) Répartition et écologie de *P. atlantica* Desf. En Algérie. Bull Soc Nat Afrique du Nord. T. 56:10–43

Monjauze A (1982) Le pays des dayas et *Pistacia atlantica* Desf. dans le Sahara algérien. Rev Forest Franc 34:277–289

Monjauze A (1980) Connaissance du bétoum *Pistacia atlantica* Desf. Biol for 4(32):356–363

Mouton JA (1967) La biométrie du limbe: mise au point de nos connaissances. Bull Soc bot Fr 123:145–158

Musset R (1953) Les régimes pluviométriques saisonniers dans l'Ouest de la France et particulièrement en Normandie. Bull Soc Linn de Normandie (1934) s8, t7: 49–65.

Quezel P, Santa S (1963) Nouvelle flore de l'Algérie et des régions désertiques méridionales. Cent Nat Rech Scient, Paris T2, Pp: 611–612.

Seltzer P (1946) Le climat de l' Algérie. Alger, Carbonel, p. 219

Stenström A, Jónsdóttir I, Augner M (2002) Genetic and environmental effects on morphology in clonal sedges in the eurasian arctic. Am J Bot 89(9):1410–1421

Stewart P (1969) Quotient pluviothermique et dégradation biosphérique. Bull Soc Hist Nat Afri Nord 59:23–36

Tirse M, Benhassaini H, Sail K, Bassou G (2014) Leaflets epidermal micro-characters of *Pistacia atlantica* Desf. subsp. *atlantica* (Anacardiaceae) under semi-arid environmental factors. Envirmn Research J 7(5):433–447

Yaaqobi A, El Hafid L, Haloui B (2009) Etude biologique de *Pistacia atlantica* Desf. De la région Orientale du Maroc. Biomatec Echo 6(3):39–49

Yang Y, Wang G, Klanderud K, Yang L (2011) Responses in leaf functional traits and resource allocation of a dominant alpine sedge (*Kobresia pygmaea*) to climate warming in the Qinghai-Tibetan Plateau permafrost region. Plant Soil 349:377–387. doi:10.1007/s11104-011-0891-y

Zohary M (1952) A monographical study of the genus *Pistacia*. Palestine J bot Jerusalem Ser 5:187–228

Zohary D (1996) The genus *Pistacia* L. In: Padulosi S, Caruso T, Barone E (eds) Taxonomy, distribution, conservation and uses of *Pistacia* genetic resources. Report of the IPGRI workshop 29–30 June 1995 Palermo, Italy

To cite this reference : El Zerey-Belaskri A. 2016. A multidisciplinary approach for the characterization of *Pistacia atlantica* Desf. subsp. *atlantica* diversity in Northwest Algeria. Doct. Thesis, University Sidi Bel Abbes, Algeria, 143p + Appendix.