



ESJ Natural/Life/Medical Sciences

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Submitted: 29 September 2020

Accepted: 13 October 2020

Published: 30 November 2020

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DOI: [10.19044/esj.2020.v16n33p189](https://doi.org/10.19044/esj.2020.v16n33p189)

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Cite as:

Lamrhari H, Ben-Said M, Jalal Z, Mehraz D,
Bouziane H (2020). Morphological and Anatomical
Characteristics of Moroccan Fir Needles in
Talassemtane National Park, North-Western Rif
Region, Morocco *European Scientific Journal, ESJ*,
16 (33), 189.
<https://doi.org/10.19044/esj.2020.v16n33p189>

Morphological and Anatomical Characteristics of Moroccan Fir

Needles in Talassemtane National Park, North-Western Rif Region, Morocco

Abstract

Moroccan fir, *Abies maroccana* Trab. forms a unique forest community in the Mediterranean basin and has a great ecological and biological values in Moroccan Rif Mountains. However, morphological and anatomical characters of the needles are poorly investigated for *A. maroccana*. This research examined the morphological and anatomical characters of Moroccan fir needles in order to determine the phenotypic needles traits of *Abies maroccana*. The study was carried out in the National Park of Talassemtane, Western Rif, Morocco. Data was collected from two-year-old needles in six stands. In each site, seven trees were selected, and 60 needles were collected from each tree. Five morphological and one anatomical characters of the needles were examined. The variance analysis (ANOVA) of quantitative needles variables revealed significant inter-tree morphological variability of needles of *A. maroccana*. Based on the morphological characters studied, the multivariate analysis (PCA) separated all trees of Moroccan fir into four groups: (A) long, thin and large; (B) long, thin and narrow; (C) short, large and thick; and finally (D) short and thick needles. As demonstrated here, the fir needles were mainly characterized by: 2 types of apex (obtuse–acute and acute needles), a broad base and the marginal resin canals. This diversity can be attributed to genetic variation and / or influence of ecological conditions.

Subject: Sciences du sol et Agriculture

Keywords: *Abies Maroccana*, Needles, Phenotypic Traits, Anatomy, Talassemtane National Park

Introduction

The Moroccan fir, *Abies maroccana* Trab., called “chouhh” in Arabic, is an endemic coniferous species of Morocco located in the mountains of Talassemtane National Park (TNP) around Chefchaouen province in the north-western Rif. *Abies marocana* is a relict species of the Tertiary (Linares, 2011) located in a refuge of exceptional moisture at its southern limit. It is listed in the IUCN Red List as Endangered species (Alaoui et al., 2011).

Currently, Moroccan fir forest covers an area of around 3760 ha whose 2988.16 ha are mixed stands (DREFLCD, 2012). The main forest species which codominate mixed stands with *A. maroccana* are represented by Atlas cedar *Cedrus atlantica* (Endl.) carrier in the upper limit of its area, Maghreb maritime pine *Pinus pinaster* var. *Maghrebiana* Huguet del Villar at the lower part with holm oak *Quercus rotundifolia* Lam., as well as Black pine *Pinus nigra* Arnold subsp. *mauretanica* (Maire & Peyer, n.d.) Heywood is also found at the lower limit of the fir forest (Benabid, 1985; M'hirit, 1990; Aafi, 2000).

The importance of this unique ecosystem is no more to be demonstrated from biological, ecological, aesthetic or cultural point of view and a great care is taken to preserve it. Several classifications have been established for *Abies* species based primarily on morphological and anatomical characteristics. *A. maroccana* belong to the Mediterranean firs which are characterized by very prickly needles and included ovuliferous bract (Gausson, 1960). From a systematic point of view, Emberger and Maire (1928) considered *A. maroccana* as a variety of the Spanish fir *Abies pinsapo* Boiss. Gausson (1952) highlighted that Moroccan fir is clearly distinguished from *A. pinsapo* by its marginal resin canals of leaves that are always flattened, while the leaves of *A. pinsapo* has marginal resin canals that are always thick. In a morphometric study of morphological diversity and structure of Moroccan and Spanish fir species, Sękiewicz et al. (2013) noted an important morphological difference between these two taxa. Moreover, recent molecular studies (Terrab et al., 2007; Dering et al., 2014) showed that Moroccan and Spanish firs have very remarkable genetic differences due to a strong influence of the Gibraltar Strait. Cosar (1946) considered Tazoat fir as a distinct species *Abies tazaotana* Cosar. However, many authors showed that the difference between the populations of Talassemtane and Tazoat is mainly due to the favorable ecological conditions in Tazoat (Benabid, 1982; Esteban & Palacios, 2007).

In Morocco, a few numbers of studies have investigated *A. maroccana*. They mainly focused, on the following aspects: Dendrochronology (Ibrahima, 2012), phytoecology (Benabid, 1982; Aafi, 2000), regeneration (Melhaoui, 1990), human disturbance impacts (Linares et al., 2011). However, researches concerning the phenotypic characterization of Moroccan fir needles are scarce (but see Sękiewicz et al., 2013). Various morpho-metric searches have

reported to be very effective in taxonomic description as well as taxa determination within species complexes (Cook & Ladiges, 1991; Passioura & Ash, 1993).

The morphological (lengths, widths, thicknesses) and anatomical (position of the resin canals in the needles, number of stomata, etc.) characters of the leaves and needles were often used as morphological markers in species description, systematics and taxonomy. (Abassi *et al.*, 2012). Many authors showed significant intra-population variability of *Pinus nigra* in Djurdjura based on needles, pollen and ovuliferous characteristics (Aidrous & Adjoud, 1992; Asmani, 1993; Abdelli, 2002).

The aim of the present paper was the determination of morphological characteristics and the position of resin canals of *A. maroccana* needles.

Materials and methods

Study area:

The study was carried out in *Abies maroccana* forest at the National Park of Talassemtane located in the western Rif Mountain, Chefchaouen province, Morocco (Fig.1). The Moroccan fir forest is restricted to the limestone substratum of western Rif, generally encountered between 1500 and 2000 m in Mediterranean humid and perhumid bioclimates. Actually, there are two blocs of Moroccan fir forest in the Rif. The bloc of Jbel Tazaot occupies around 300 ha, and appears on its northern slope. Located south of the Tazaot, the Talassemtane bloc extends over approximately 3760 ha where Moroccan fir always remains dominant, on the northern, eastern and western slopes.

In the upper limit of its area, fir forest is progressively replaced by Atlas cedar, while the Maghreb maritime pine dominates the lower part with the holm oak. Black pine is also found at the lower limit of the fir in small patches (Benabid, 1985; M'hirit, 1990; Aafi, 2000). Substratum dominated by limestone and dolomite. The soil is classified as Alfisols in association with Mollisols (Benjelloun, 1993). The annual rainfall varies between 500 mm in the eastern valleys and can exceed 1500 mm on the high mountain peaks (Lakrâa 2156 m). The snow is less important (Benabid, 2000). In the Moroccan fir forest, the mean temperature is around of 12-14 °C, the averages of the maxima temperatures of the hottest month (August) did not exceed 33 °C. The averages of the minima temperature of the coldest month are of the order of 0 °C and can reach - 3 °C at high altitude (Ghallab & Taiqui, 2015).

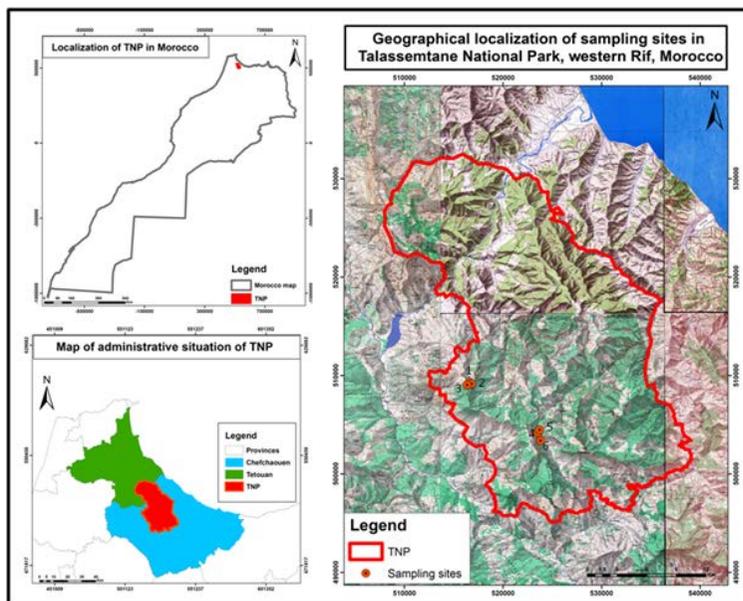


Figure 1. Geographical localization of sampling sites in Talassemtane National Park, western Rif, Morocco.

Data collection:

In this study, we sampled six sites in the Talassemtane National Park (Tab. 1), within each site we randomly selected seven Moroccan fir trees (a total of 42 individuals).

Table 1. Characteristics of the six studied *A. marocana* stands

Sites	Species	Elevation (m a.s.l.)	Latitude (N), Longitude (W)	Precipitation (mm)	Temperature (°C)	
					Max	Min
1	<i>Abies marocana</i>	1748	35°11'7.52"N, 05°13'2.29"W	630,29	22,78	13,25
2		1754	35°11'3.91"N,05°12'51.73"W		22,87	
3		1658	35°11'22.37"N,05°13'15.80W		22,64	
4		1717	35°8'23.77"N, 05°8'23.36"W		22,78	
5		1789	35°8'2.17"N, 05°8'22.06"W			
6		1713	35°8'33.46"N, 05°8'15.83"W			

Max: maximal; Min: minimal.

From each tree, sixteen two-year-old needles were collected from northern parts of the crowns at a same height of 1.30 m. All plant material was put in bags bearing tree number and kept cool and transported to the

laboratory. A total of 2520 needles were measured and characterized. The sampling was carried out during the vegetative period of 2018.

Three Morphological traits (length (L), width (W) and thickness (T)) were measured using sliding caliper. The width (W) and thickness traits were measured from the central part of the needle (e.g., 25–75% of the needle length). In addition, the ratios (length to the width ratio (L/W) and width to the thickness ratio (W/T) were calculated. The type of needle apex (obtuse-acute (a); acute (b); apiculate (c); obtuse (d)), the base of the needle (broad (a) , straight (b)) (Fig. 2) and the position of the resin canals (marginal (a); median (b)) were determined.

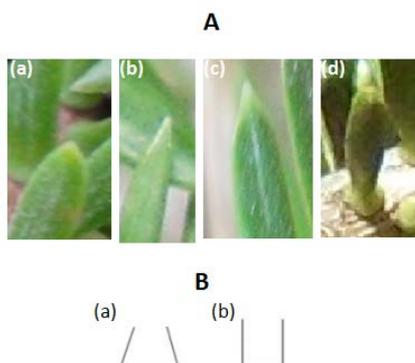


Figure 2. Type of needle apex **A:** obtuse-acute (a); acute (b); apiculate (c); obtuse (d) and **B:** type of needle base (broad (a) and straight (b)) of *Abies maroccana*.

The anatomical sections were obtained by depositing each needle between two polyester blocks and making fine cross sections at the level of the middle part of needles (25- 75% of its length) using a razor blade, then the obtained sections were colored by safranin and fast green (double staining).

The sections were finally mounted between slides and plates in a drop of water, three to four sections were taken perslide and observed under an optical microscope (Olympus).

Data analysis:

The Kolmogorov Smirnov test was used to verify the normality of the data. The basic statistics (mean, standard deviation, minimum and maximum values and variation coefficient) for trees were calculated. To assess the variation of the needles morphological characters among individuals, one-way analysis of variance (ANOVA) was used. The Pearson's correlation coefficient was used to assess the bilateral dependencies existing between the different quantitative variables of the needles. In order to establish the relationships between trees, multivariable statistical analysis was used, namely principal component analysis (PCA). For this purpose, the following variables

were included: length (L), width (W), thickness (T), the length to the width ratio (L/W) and the width to the thickness ratio (W/T).

All statistical analyses were performed using IBM SPSS Statistics 20.0 <https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-20>.

Results

Biometric analysis:

The average length (L) of needles varied between 1.22 mm and 19.52 mm with an average of 10.18 mm for all sites (Tab. 2). The average value of the needles width (W) was 1.88 mm (range 0.38 - 2.95 mm). The thickness (T) of needles varied between 0.21 and 2 mm with an average of 0.54 mm. Moreover, for the L/W ratio, the minimum and the maximum values were 0.55 and 25.24 mm, respectively, with an average of 5.52 mm. The W/T ratio varied between 0.21mm and 36.33 mm with an average of 3.73 mm. Finally, the coefficient of variation of all sites characters varied widely between sites particularly for the width character, indicating a high variability inter-sites (Tab. 2). Variance analysis of the studied quantitative variables of *A. maroccana* needles showed high significant differences among trees (Tab. 2).

Table 2. Statistical description of the five characters studied.

Characters	Statistics	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Mean
L (mm)	Mean	9.91±2.	9.71±1.	9.85±1.	11.12±2	11.73±2	8.78±1.	10.18±2
	± SD	14	65	42	.22	.90	42	.25
	Min-	4.52-	5.90-	6.39-	1.29-	5.69-	1.22-	1.22-
	Max	15.91	13.68	13.84	16.48	19.52	12.48	19.52
	CV	21.59	16.99	14.42	19.96	24.72	16.17	22.07
	F- test	107.26*	47.86**	72.3***	104.35*	208.11*	68.31**	128.08*
		**	*		**	**	*	**
W (mm)	Mean	0.87±0.	1.71±0.	2,25±0,	1,87±0.	1,91±0.	1,70±0.	1,88±0.
	± SD	21	25	21	95	27	13	28
	Min-	0.3-2.47	0.46-	1.58-	1,18-	1,12-	1,23-	0,38-
	Max	24.14	2.23	2.95	17.9	2.92	2.03	2.95
	CV	27.62**	14.62	9.33	50.8	14.14	7.65	15.03
	F- teste	*	23.81**	44.27**	2.48***	123.13*	27.23**	112.13*
		*	*		**	*	**	
T (mm)	Mean	0.54±0.	0.49±0.	0.76±0.	0.44±0.	0.5±0.1	0.52±0.	0.54±0.
	± SD	13	14	16	15	3	1	17
	Min-	0.29-	0.21-	0.43-	0,22-2	0.24-	0.35-0.9	0.21-2
	Max	1.01	1.06	1.24	34.09	1.05	19.23	31.65
	CV	24.07	28.57	21.05	54.7***	26	30.03**	110.18*
	F- test	30.44**	61.4***	62.313*		118.33*	*	**
	*		**		**			

L/W (mm)	Mean	5.36±1.	5.84±1.	4.42±0.	6.20±1.	6.17±1.	5.15±0.	5.52±1.
	± SD	47	70	83	38	41	80	45
	Min-	2.77-	3.14-	2.53-	0.79-	3.27-	0.55-	0.55-
	Max	25.18	25.24	7.41	10.21	12.11	7.67	25.24
	CV	27.43	29.11	18.78	22.26	22.85	15.53	26.26
F- test	24.59**	22.09**	81.10**	61.8***	97.41**	25.4***	58.56**	
		*	*	*		*	*	
W/T (mm)	Mean	3.71±1.	3.70±0.	3.09±0.	4.45±1.	4±0.86	3.45±1.	3.73±1.
	± SD	39	90	64	19	1.97-	73	25
	Min-	0.21-	0.98-	1.82-	0.77-	8.14	1.86-	0.21-
	Max	25.44	6.60	4.89	8.73	21.50	36.33	36.33
	CV	37.47	24.32	20.71	26.74	50.6***	50.14	33.43
F- test	7.82***	37.15**	54.13**	119.98*		5.41***	30.8***	
		*	*	**				

SD: standard deviation; Min: minimum; Max: maximum; CV: Coefficient of Variation;
Statistical significance: ***: $p < 0.001$.

Statistical analyses:

The results of correlation analyses showed that needle length was positively correlated ($p < 0,01$) to the W/T ratios, while it was negatively correlated to thickness (Tab. 3). Furthermore, the needness width was positively correlated to the thickness ($p < 0.01$).

Table 3. Correlation matrix of the quantitative needles variables (length (L), width (W), thickness (T), length to the width ratio (L/W) and width to the thickness ratio (W/T)) according to Pearson's correlation coefficient.

*,	Variables	L	W	T	L/W	W/T
	L	1				
	W	0.287	1			
	T	-0.327*	0.650**	1		
	L/W	0.802**	-0.312*	-0.737**	1	
	W/T	0.606**	-0.193	-0.837**	0.760**	1

correlation is significant at the $p < 0.05$ level; **, correlation is significant at the $p < 0.01$ level.

Table 4 showed the eigen values and loading factors of the first PCA axes. PCA axes 1 and 2 explained 56.83% and 34.97% of the total variance (Tab. 4). On the first component axis, the main structuring characters were the length, the L/W ratio and the W/T ratio in the positive direction and thickness in the negative direction. On the second component axis, the discriminates characters were width and thickness.

The ordination of the 42 trees measured according to the first two PCA axes is shown in figure 3.

Table 4. Loading factors and eigen values of the first two PCA axes
 Performed for needless quantitative variables.

Quantitative needles variables	PC1	PC2
Length	0.918	0.330
Width	0.019	0.969
Thickness	-0.650	0.730
Length/Width	0.914	-0.270
Width/Thickness	0.861	-0.308
Eigen values	2.84	1.75
Explained variance (cumulative %)	56.83	91.80

The quantitative variables with the largest participation in each component are distinguished in bold.

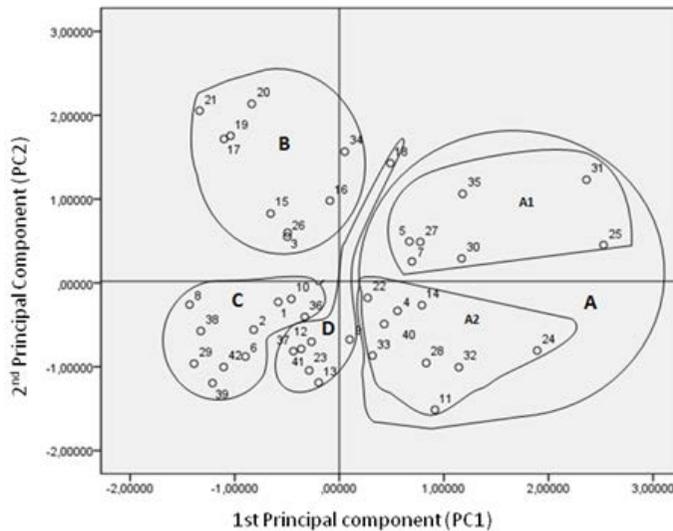


Figure 3. Scatter plot of the 42 trees of *A. maroccana* based on the two first PCA axes.

The PCA performed for all trees allowed the separation of the 42 *A. maroccana* trees in 4 groups according to their needles morphological characters (Fig.3). Group A appeared close to the positive direction of the PC1 and characterized by long and thin needles as well as by high length to width ratio and width to thickness ratio. Furthermore, group A was subdivided in two subgroups (A1 and A2) according to tree needle width, where trees of subgroup A1 had wide needles whereas those of subgroup A2 had narrow needles. Group B was distinguished by wide and thick needles. Conversely to the subgroup A1, we identified a group C characterized by trees with short and

thick needles, as well as by low L/Wand W/T ratios. Finally, Group D appeared to be intermediate between A, B, and C groups (Fig. 3).

In terms of the types of apex, *A. maroccana* trees presented mainly obtuse–acute (42 %) and acute needles (35 %) (Fig. 4A). The resin canals were almost marginal (72%) and located in the mesophyll, adjacent to the epidermis, sometimes, they are in the mesophyll in the median part of the needle (28%) (Fig. 4C, 5).For the overall trees examined in this study, Moroccan fir needles showed exclusively a base broad and not straight (Fig.4B).

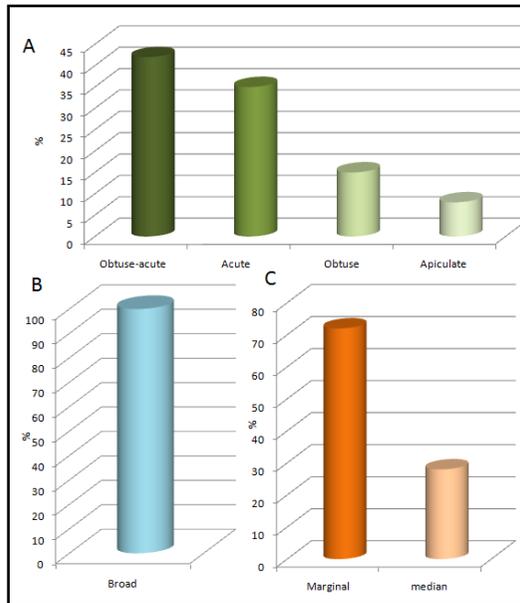


Figure 4. Percentages of the type of needle apex A, type of needle base B and position of resin canals C of *Abies maroccana*.

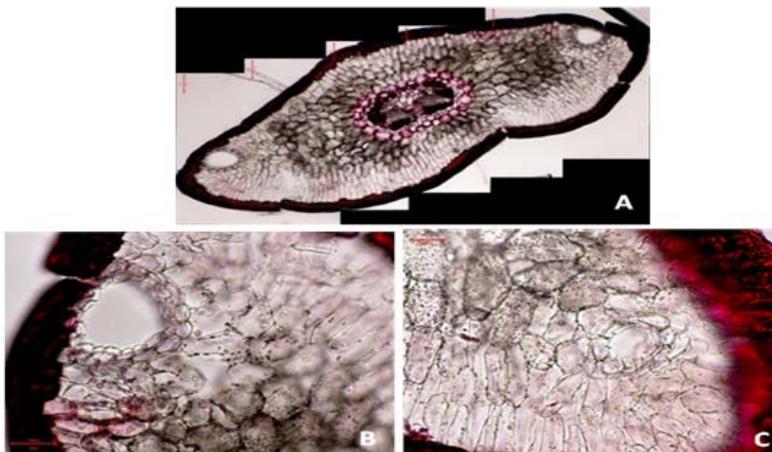


Figure 5. A. Anatomical needle section of *Abies maroccana*. B. marginal position of resin canals. C. median position of resin canals

Discussion

The present study aimed to characterize different phenotypes of *Abies maroccana* needles in the National Park of Talassemtane through the examination of morphological traits and position of resin canals.

Melhaoui (1990) reported that the length of *A. maroccana* needles ranged from 5 to 15 mm and their width from 2 to 2.5 mm. However, our biometrical analysis showed slight differences. We found that needles length of the studied fir trees varied between 1.22 and 19.52 mm, width 0.38 and 2.95 and thickness 0.21 and 2 mm. The coefficient of variation indicated that the majority of the morphological traits showed significant inter-sites variability.

The variance analyses showed highly significant differences among the studied trees suggesting a high degree of heterogeneity. Several authors have reported similar results in several *Pinaceae* species (Arbez & Millier, 1970; Aussenac, 1973 – 1990; Destremau, 1976; Calamassi & Falusi, 1990; Illoul & Moualek, 1991; Aidrous, 1992; Satour, 1992; Abdelli, 2002; Dangasuk & Panetsos, 2004; Ait said et al., 2005; Allilou et al., 2006; Wahid, 2006; Liesche et al., 2010; Bilton et al., 2010; Kenichi, 2010; Abassi et al., 2012; Diop et al., 2013). Morphological differences observed between individuals belonging to the same species reflect different responses to environmental conditions (Lodé, 1998).

The results of correlation analyses showed that needle length was negatively correlated to thickness, whereas needle width was positively correlated to thickness. Bouzina (2016) found that long needles are frequently wide but not necessarily thick in *Abies numidica* from Algeria. The author reported that the thick needles had an importance mesophyll.

The result of Principal Component Analysis grouped the overall trees of *A. maroccana* in four groups (A, B, C, and D) according to the similarity of their morphological needles characters (length, width, thickness, length to width ratio and width to thickness ratio). Trees within each group appear to share a similar morphological variation, which is probably related to their common gene-pool and/or to the fact that they are subjected to the same local environmental conditions. Indeed, Aussenac et al., (1990) reported that the dimensions of needle trees vary according to their position towards the light. Trees belonging to the subgroup A1 and group B had long, wide and thick needles, respectively. The needles of those trees have been collected from the lighted branches. The lighted branches have longer, wider and thicker needles than the shadow branches in *Abies nebrodensis* trees, suggesting that light conditions influenced needle characters (Bottacci et al., 1990). Indeed, Alilouet al., (2006) observed under pollution stress some changes in the length, width and density of stomata of Argan leaves from Morocco. However, as our study area occurs in high mountains there is no source of any kinds of pollution.

Most of trees constituted group B belong to the stand 3 located at 1658 m and had slight needles morphological variability compared to the remaining stands (1, 2, 4, 5, 6) situated between 1713 and 1789 m. Various topographic and climatic factors are reported to influence morphological traits of trees. Altitude, minimum temperature and aridity influenced the morphological variability of many provenances of *Pistacia atlantica* (Belhadj et al., 2008; Ait Said, 2011), *Eucalyptus* (Franks et al., 2009) and some species of *Banksia* genus (Drake et al., 2013). As stated by Hafsi et al., (2017), environmental factors, mainly continental gradient and winter thermal stress, relative to the moderating effect of altitude, affected the phenotypic variation of *Juniperus oxycedrus* from Algeria. However, ecological conditions may not be sufficient to cause significant variation for all individuals in a population (Aussenac, 1973). Kaced and Aimen (1998) who studied the morphological characters of needles and twigs of *Cedrus atlantica* from Algeria showed that the length of the needles, the horny part of the apex and the mesoblast varied among trees within the same site.

Sekiewicz et al., (2013) stated that all types of needle apex (obtuse-acute; acute; apiculate; obtuse) were present in *A. maroccana* with a similar contribution. However, our result showed that the *A. Moroccan* trees were characterized mainly by obtuse-acute and acute needles. Moreover, Sekiewicz et al., (2013) showed that *A. maroccana* needles were characterized by two types of base (broad and straight), whereas we found only broad base for all trees.

Several authors reported that Morocco fir is characterized by the marginal position of the resin canal (Liu, 1971; Vidaković, 1991; Farjon, 1990). Our results based on anatomical sections of *A. maroccana* needle indicated that the resin canals were almost located in marginal position (72%), and in a lesser extent, in median position. These results support those of previous studies. In the case of thick needles, the distance between resin canals and epidermis was larger while fine needles had mostly resin canals in contact with their walls, this may be explained by the importance of mesophyll in the thick needles (Bouzina, 2016).

An earlier study which examined the variation in the position of resin canals in the needles of *Abies* species (from Spain, USA, Turkey) and provenances, revealed intra-population variability from the same geographical area and even within the same sample site (Panetsos, 1992). This variability depends on the position of the shoots from which the needles were collected. In the present study, the needles were taken from very similar parts of the crown so that the differences observed were not random. The variability in the position of the resin canals appears to be under genetic control and not as much affected by ecological factors (Roller, 1966).

Moroccan fir trees grow in a dolomitic limestone substratum, either in deep or superficial soils, under humid and perhumid bioclimates, with cold, exceptionally cool or very cold variants (Aafi, 1995). This ecological variability could be responsible for the morphological traits heterogeneity of *A. maroccana* needles. The influence of certain local environmental conditions, or genetic variation, or a combination of these two factors, could be responsible for the observed morphological variability within and among populations (Willan, 1985).

Conclusion

The present morphometric study has revealed significant inter-trees variability of *Abies maroccana* needles. Indeed, results showed that the needles of studied *A. maroccana* trees had various forms: (i) long, thin and large; (ii) long, thin and narrow; (iii) short, large and thick; and (iv) short and thick needles. Moreover, the thick needles found to have a very developed mesophyll layer. Moroccan fir needles have characterized mainly by 2 types of apex: obtuse–acute and acute needles, with broad base and marginal resin canals. This variability can be explained by the influence of some ecological conditions, or a genetic variation, or a combination of these two factors.

Acknowledgements

The authors thank La Direction Provinciale des Eaux et Forêts et à la Lutte Contre la Désertification de Chefchaouen and Le Haut-Commissariat aux Eaux et Forêts et à la Lutte Contre la Désertification du Rabat. We also want to thank very particularly the support of Abdelaziz DOUIER, (director of HCEFLCD Chefchaouen) at the time of the development of the research, and Mohamed JABRAN, (director of Talassemrane National Park Chefchaouen), Ahmed DAOUDIA, Chief of Centre de Conservation et de Développement des Ressources Forestières Chefchaouen), who provided guided access to the forests of Sidi Abdelhamid and Talassemrane. We also thank Dr. Jalal KASSOUT for his precious laboratory assistance. We would like to express our deepest appreciation to Mr. Mohamed Ater professor in the Faculty of Science of Tetouan, Abdelmalek Essaadi University, for allowing us to carry out our work in his Applied Botanical Laboratory.

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