



Asian Journal of Plant Sciences

ISSN 1682-3974

science
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Anatomy, Histochemistry and the Biometrics of Fibres of *Chamaerops humilis* L. Growing in Two Different Localities in Algeria

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Abstract: The anatomical and the histochemical study of the, young, adult rachides and leaflets of *Chamaerops humilis* L. region of Tlemcen and Oranian littoral shows that the general structure is similar to that of the majority of Arecaceae. This species presents cellulosed and lignified cell walls. Coniferyls lignins miss in the cell walls of abaxial fibres of the rachides and leaflets, also the inner bundle-sheath fibres are deprived of coniferyls lignins. On the other hand, syringyls lignins are abundant in tissues of the rachides and the leaflets of the two regions. The abaxial fibres of the leaflets of Tlemcen are continued in direction of bundle-sheath fibres, these walls are thicker than the region of littoral. The vascular bundles are continue («open type») of the sample of Tlemcen and discontinue («closed type») in that of the littoral region. The biometric study of fibres of rachides and leaflets of the *chamaerops* of Tlemcen shows that the average length of its fibres is 0.46 mm for the young rachides and 0.55 mm for the adult, those of the leaflets is 0.63 mm for the young and 0.66 mm for the adult leaflets. The comparative test shows that there is not significant difference within the species of Tlemcen. The values lengths obtained make it possible to classify this species among the plants with short fibres, this characteristic brings closer it with the plants with paper interest like the esparto. As for the species of the Oranian littoral, the lengths found for the young and adult rachides are 0.77 m and 1.28 mm, respectively the comparative test has shown that there is not significant difference for its fibres, on the other hand, there exist a difference for the leaflets of this region since T calculated is higher than T threshold. The average lengths of this species make it possible to bring it closer with the plants with average fibres. The comparative study was also carried out between the organs of the two regions, the results showed that there is a significant difference between the young rachides, adult rachides and adult leaflets of the two regions between then, these results make it possible to suggest that these species probably come from two different populations and that can be one is more advanced than the other.

Key words: Palms, anatomy, histochemistry, lignins, fibres, *Chamaerops humilis* L

INTRODUCTION

Chamaerops humilis L. is a perennial monocotyledone pertaining to the family of Arecaceae (Tomnilson, 1961) generally called dwarf palm tree or palm tree of the Mediterranean, this species is very widespread in the Mediterranean countries where it represents a major biological indicator of the stage of thermo vegetation Mediterranean (Medail and Quezel, 1996). In Algeria, it extends on an important in the arid regions and semi-arid area.

Its apparatus vegetative with aspect in tuft is characterized by a feather-grass runs not ramified, the leaves are in range, palmatifid, rigid, glaucous, silver plated, are covered with squamiform and persistent hairs

(Maire, 1957), its leaves are rich in fibres. As well as fibres of the *Stipa tenacissima* L. *Lygeum spartum* L. and *Aristida pungens* Desf., the foliar fibres of *Chamaerops humilis* L. can constitute an interesting material in industries of cellulose.

With its adventitious system racinaire drawing, the *chamaerops* stabilize the soil against any scouring due to a hydrous or wind erosion.

Because of its great rusticity, of its capacity of recovery after the fire, le *chamaerops* allows the maintenance of a certain humidity (Bouazza *et al.*, 2001).

Works on the *chamaerops* of Algeria are very few, this studies concern an anatomical and histochemical of tissues of palms and biometric study of fibres of this species coming from two various Algerian localities.

MATERIALS AND METHODS

The samples which were the subject of this study are fresh leaflets and rachides taken from young (having a maximum of 6 leaves) and adult tufts (having more than 100 leaves) of *Chamaerops humilis* L growing in two regions of Algeria.

The region of Tlemcen (T) is characterized by a higher semi-arid climate defined by a hot dry summer and a cold winter. Its altitude is of 850 m. The latitude is of 34°53' N and longitude is of 1°14' O. The soil has an argillaceous texture. The average annual temperature is of 17.8°C and the annual precipitation are 487 mm an⁻¹.

The region of the Oranian Littoral (OL) is characterized by a very wet climate, Mediterranean, its altitude is 200 m, the soil is sandy-limestone. The latitude is of 35°44' N and longitude is of 0°33' O. The annual temperature average 18.8°C and the precipitations average is of 312 mm an⁻¹.

Anatomy and histochemistry: Fresh transverse sections were cut by hand with a razor blade from the material described above.

The anaomical study was carried out by double coloring described by Langeron (1934). This coloring allows the description of the vegetable wall; the cellulosic cell walls are coloured in red by the congo-red and lignified ones in green by the Methy-green.

Lignin was stained with wiesner regents (Alder *et al.*, 1948). Were phyloroglucinol in acidic conditions gives a red-pink product with mainly the cinnamaldehyde groups present in lignin it gives a purple-red coloration (Gibbs, 1958).

After colouring, the sections are observed and photographed using a photo microscope of the ZEISS.

Morphology and biometrics of fibres

Dissociation and colouring: Fragments of young and adult leaflets and rachides of 0.5 cm length are placed in tubes containing an acetic mixture of acid and hydrogen peroxide with equal volume. They are put at the oven drying at 70°C during one week. Acetic acid has for role to dissolve existing pectic cement in the average plate. After dissociation of the mixture, the fibres are coloured by safranin during one week.

The red colouring which appears explains the lignification of tissue. A suspension of cell is taken using a Pasteur pipette deposited between blade and plate then observed under the photonic microscope.

Measurement of fibres and statistical test: The length of 30 fibres was given using an ocular micrometer. The values obtained were compared using the statistical test to check if there is or not a variability.

Calculation of the average X:

$$\bar{X} = \frac{\sum_{i=1}^n X_i}{n}$$

X_i : Characteristic of the sample.

n : Cuts or number of sample.

Calculation of the variance S:

$$S^2_k = \frac{\sum_{i=1}^{n_k} X_i^2}{n} - \bar{X}_k^2, k=1, 2, \dots, 8.$$

S₁, S₂, S₃, S₄ are the variances of the young and adults rachides, young and adult leaflets of Tlemcen.

S₅, S₆, S₇ and S₈ are the variances of the young and adult rachides s, of the young and adult leaflets of the Oranian Littoral.

Calculation of T: (Test of Student-Fisher)

$$T_1 = \frac{\bar{X}_2 - \bar{X}_1}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

T₁ = The value of T calculated for the young and adult rachides (T₂ is the value of T calculated for the young and adult leaflets) of Tlemcen.

T₃ = The value of T calculated for the young and adult rachides (T₄ is the value of T calculated for the young and adult leaflets) of the Oranian littoral.

T₅ = The value of T calculated for the young rachides (T₆ is the value of T calculated for the adult rachides) of the two sampling regions.

T₇ = The value of T calculated for the young leaflets (T₈ is the value of T calculated for the adult leaflets) of the two sampling regions.

This expression follows the law of Student and Fisher it is the comparison between the value T observed and the value threshold given in the table T according to the number of degree of freedom d.d.l and the selected risk (α = 5%).

If T observed > T threshold, there is a significant difference.

If T observed < T threshold there is not a significant difference.

T threshold = 2

RESULTS

Anatomy: Young and adult rachides. The observations carried out in transversal sections of young and adult rachides show of the exterior towards the interior.

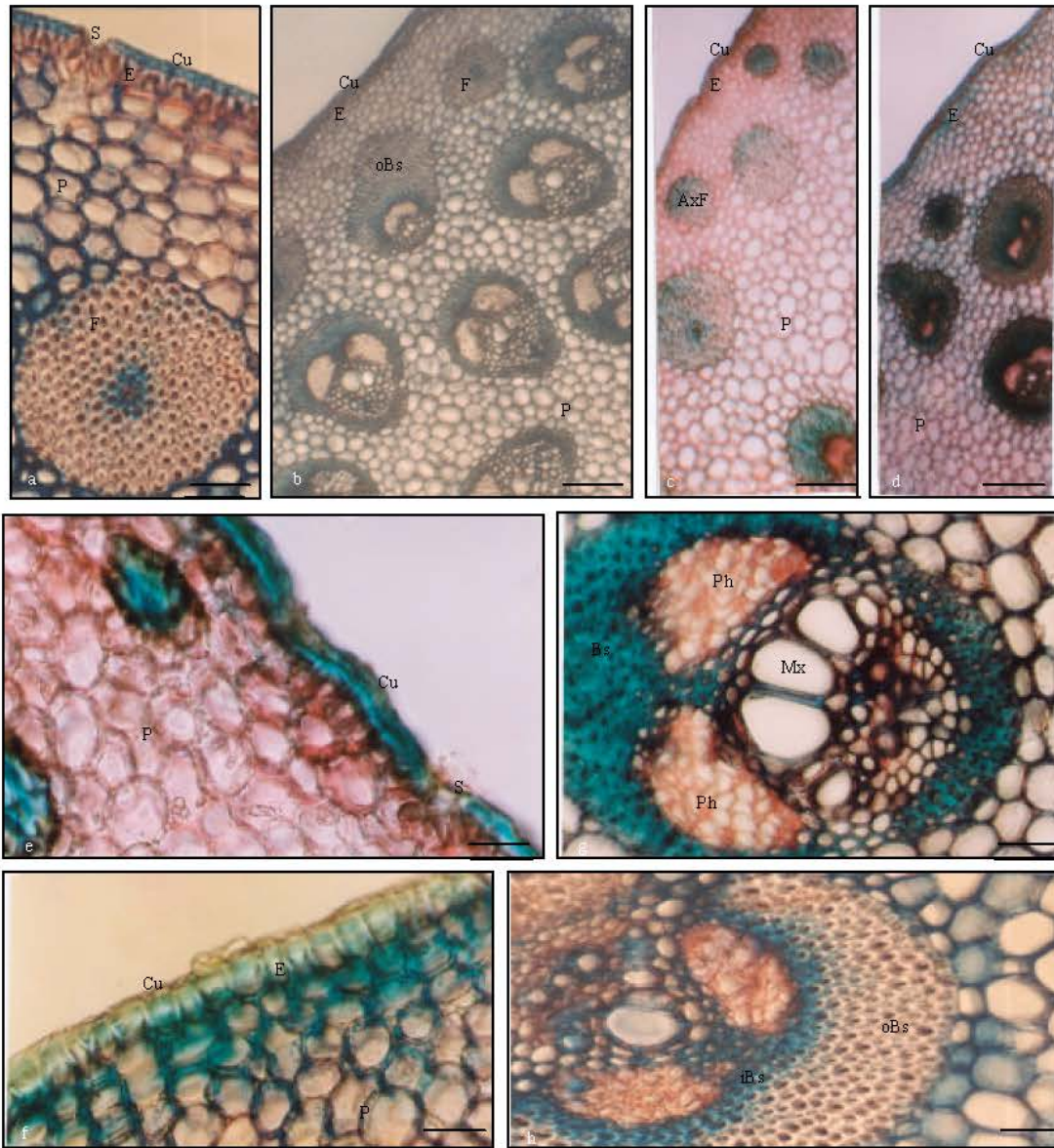


Fig. 1: (a) Detail of a young portion of rachides of Tlemcen. X 2100. Epidermis covered with cuticle introrped by stomata. A fibre which will probably developed in vascular bundle, (b) Overall picture of an adult portion of rachides of Tlemcen. X 525. Increase in the size of the cells of the parenchyma of the periphery towards the center, (c) Overall picture of a young portion of rachides of the Oranian littoral. X 525, (d) Overall picture of an adult portion of rachides of Tlemcen. X 525, (e) Young detail of rachides of the Oranian littoral, epidermis thickened by a layer of Cuticule. X 2100, (f) Adults detail of rachides of the Oranian littoral showing the lignification of the cells of epidermis. X 2100, (g) Detail of a vascular bundle of the adult rachides of the showing littoral: The lignification of the xyleme and the bundle-sheath, X 2100, Presence of 5 metaxylemes and (h) Detail of avascular bundle of the young rachides of showing Tlemcen: The beginning of lignification in the inner bundle-sheath. X 2100, Presence of only one metaxyleme. The bar on micrographs represents: 5 μ m (Fig. 1a, e, f, g and h); 10 μ m (Fig. b and c); 25 μ m (Fig. d) Cu: cuticle; E: Epidermis; H: Hypoderme; S: Stomata; F: Fibres; AxF: Abaxial fibre; Me: Mesophyll; Bs: Bundle-sheath; iBs.: Inner bundle-sheath; oBs.: Outer bundle-sheath; Ph: Phloem; X: Xylem; Mx: Metaxylem; Vb: Vascular bundle; P: Parenchyma; Pp: Palisade parenchyma (+): Positive reaction; (-): Negative reaction; (++) : Contrast

- Epidermis: Formed of a base of cellulose cells walls and which lignify at the adult rachides. It is interrupted by stomata and is covered by a thick cuticule (Fig. 1a-d).
- Abaxial Fibres: They are laid out in small solid masses rounded located right under the epidermis, the walls cellulosed and are lignified in the center at the young rachides taken from the region of Tlemcen (Fig. 1a and b) That of the region of the littoral is characterized by lignified fibres at the young and adult rachides (Fig. 1c and d).
- Parenchyma: consists of round and jointed cells with cellulosed walls (Fig. 1b, c and e), their sizes are increasing while going from the epidermis towards the center of the section (Fig. 1b).
- Vascular bundle: It is typical of palm tree, consisted a conducting part and a fibrous part. In the young rachides of Tlemcen, the inner and outer peripheral sheaths are made of fibres with walls lignified on the level of the basal part only (Fig. 1h).

The Vascular bundle increase by size of the periphery towards the center of the section.

The xylem is made of 3 to 4 vessels of metaxylemes in the adult rachides (Fig. 1g) and 1 to 2 in the young rachides (Fig. 1h). The phloem consists of two solid masses of phloem surrounded of fibres with lignified walls (Fig. 1g and h).

Young and adult leaflets: Transverse sections realized in young and adult leaflets show that the limb is made up:

- Epidermis: It self made up of cells with cellulose wall doubled with dimensions external of cuticule (Fig. 2b) in the adult the cells are lignified (Fig. 2c).
- Hypoderme: located in lower part of the epidermis, made of a layer of lengthened cells with cellulose walls (Fig. 2b and c).
- Palisade parenchyma: made of very lengthened cells of rectangular form which border the fibrous solid masses, these cells are with wall thick, slightly lignified and equipped with chloroplasts. This tissue is quite visible in the young and adult leaflets of the species of Tlemcen but little developed in that of the littoral (Fig. 2b and c).
- Mesophyll: made of jointed cells with thin and cellulose walls equipped rich in chloroplasts (Fig. 2b and c).
- Abaxial Fibres: are different sizes and are made of cells walls thick and lignified at the adult (Fig. 2c), cellulosed at the young leaflets of the two localities (Fig. 2a and b). Certain fibres are prolonged in direction of the vascular bundle (Fig. 2c).

Table 1: Description of conyferyls lignins at the young and adult rachides of *Chamaerops humilis* L. of the two sampling regions

Localities		Bs						
		E	AxF	iBs	oBs	Ph	X	P
Young Rachides	T	-	-	++	-	-	+	-
	OL	-	-	+	+-	-	+	-
Adult Rachides	T	-	-	++	++	-	+	-
	OL	-	-	+	+-	-	+	-

T: Tlemcen; OL: Oranian Littoral; E: epidermis; AxF: abaxial fibre; Bs: Bundle-sheath; iBs: inner Bundle-sheath; oBs: outer Bundle-sheath; Ph: phloem; X: xylem; P: parenchyma; (+): positive reaction; (-): negative reaction; (++) contrast

Table 2: Description of conyferyls lignins at the young and adult leaflets of *Chamaerops humilis* L. the two sampling regions

Localities		Bs						Me	
		E	H	AxF	iBs	oBs	Ph		X
Young leaflets	T	-	-	-	+	+-	-	+	-
	OL	-	-	-	+	+-	-	+	-
Adult leaflets	T	-	-	-	++	++	-	+	-
	OL	-	-	-	+	+-	-	+	-

T: Tlemcen; OL: Oranian Littoral; E: epidermis; H: hypoderme; AxF: abaxial fibre; Me: mesophyll; Bs: Bundle-sheath; iBs: inner Bundle-sheath; oBs: outer Bundle-sheath ; Ph: phloem; X: xylem; (+): positive reaction; (-): negative reaction; (++) contrast

Table 3: Description of syringyls lignins with at the young and adult rachides of *Chamaerops humilis* L. of the two sampling regions

Localities		Bs						
		E	AxF	iBs	oBs	Ph	X	P
Young rachides	T	-	+	++	+	-	+	-
	OL	-	+	+	+	-	+	-
Adult rachides	T	-	+	++	+	-	+	-
	OL	-	+	++	+	-	+	-

T: Tlemcen; OL: Oranian Littoral E: epidermis; AxF: abaxial fibre; Ph: phloem; X: xylem; P: parenchyma; Bs: Bundle-sheath; iBs: inner Bundle-sheath; oBs: outer Bundle-sheath (+): positive reaction; (-): negative reaction; (++) contrast

- Vascular bundle: Is discontinue («open type») at the sample of Tlemcen (Fig. 2h) and continue («closed type») at that of Oranian littoral (Fig. 2i), these tissues surrounded by a bundle-sheath whose fibres are with walls strongly lignified at the adult leaflets compared to the young leaflets.

The Vascular bundle of the median part are lignified than those of the apical part.

Histochemistry

Description of conyferyls lignins: At the young rachides of Tlemcen, the only inner bundle-sheath cells react positively, at the adult rachides of the littoral the inner and the outer bundle-sheath cells are positive with the reaction.

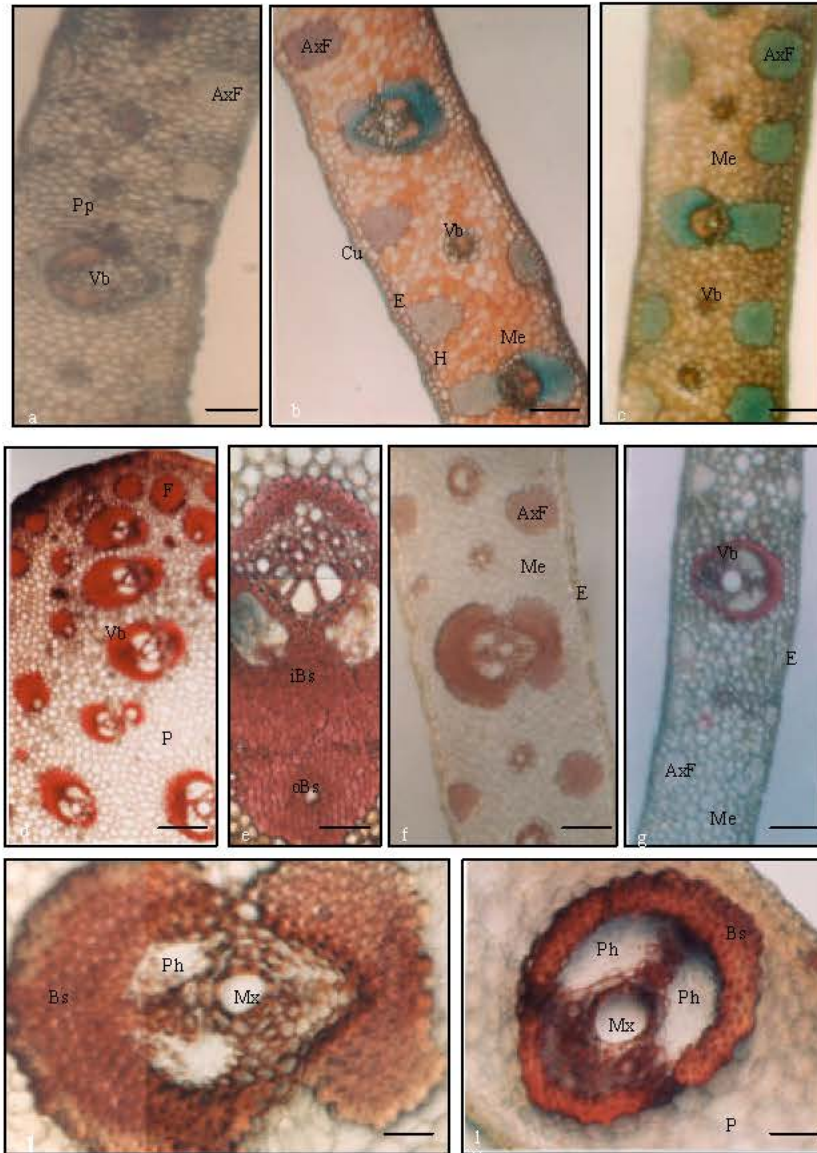


Fig. 2: (a) Overall picture of a young portion of leaflets of Tlemcen. The abaxial fibres are deprived of lignins. X525, (b) Overall picture of a young portion of leaflets of the littoral. The abaxial fibres are deprived of lignins. X525, (c) Overall picture of an adult portion of leaflets of the littoral X 525, (d) Overall picture of a young portion of rachides of the littoral. Presence of syringyls lignins in abaxial fibres, the bundle-sheath and in xyleme. X525, (e) Detail of a vascular bundle showing the abundance of syringyls lignins at adult rachides of Tlemcen. X 2100, (f) Overall picture of an adulte portion of leaflets of Tlemcen, showing: Abundance of syringyls lignins in abaxial fibres, inner and outer bundle-sheath and the xylème; Appearance of the future vascular bundle. X525, (g) Overall picture of an adult portion of leaflets of the Oranian littoral, showing them conyferyls lignins only in the bundle- sheath. X 525, (h) Detail of a vascular bundle of type open in an adult leaflets of Tlemcen The inner an douter bundle-sheaths and the xylème contain syringyls lignins. X 2100 and (i) Detail of a vascular bundle of type closed in an adult leaflets of the littoral. The inner and outer bundle-sheaths and the xylème contain syringyls lignins X 2100. The bar on micrographs represents: 5 µm (Fig. 2e, h and i); 10 µm (Fig. 2a, b and c); 25 µm (Fig. 2d and e); Cu: cuticle; E: epidermis; H: Hypodermis; S: Stomata; F: Fibres; AxF: Abaxial fibre; Me: Mesophyll; Bs: Bundle-sheath; iBs: Inner bundle-sheath; oBs: Outer bundle-sheath; Ph: Phloem; X: Xylem; Mx: Metaxylem; Vb: Vascular bundle; P: Parenchyma; Pp: Palisade parenchyma (+): Positive reaction; (-): Negative reaction; (++): Contrast

The reaction is positive in the walls of the sheath of the adult rachides of the two regions. At the young rachides of the Oranian littoral, the external part of the bundle-sheath reacts slightly on the other hand, the internal and strongly reactive part (Table 1). The reaction is positive in the walls of the sheath of the adult rachides of the two regions.

The lignification of the young rachides and adults of the littoral is more important in the basal parts than in the apical parts.

In the young and adult leaflets of the two regions, the cell walls of bundle-sheath and the xylem react positively (Table 2) the axidal fibres are devoided of coniferyls lignins (Fig. 2a and g).

We observe a difference of reactivity in the outer bundle-sheath cell walls of Tlemcen.

The reaction to hydrochloric phloroglucinol is positive on the level of the bundle-sheath like in the xylem of the young and adult leaflets. This reaction is very strong in the inner sheath of the adult leaflets. And is accentuated while going from the apical part towards the basal part of the young and adult leaflets.

Description of syringyls lignins: The reaction of Maüle is strong in the majority of lignified tissues. In the young rachides of Tlemcen, a difference in intensity of the reaction is visible in the walls of the inner and outer bundle-sheath cells. The apical part is negative, the median answers slightly better and the basal part answers in all the inner and outer bundle- sheath (Table 3).

In the adult rachides of the littoral, the reaction is very strong in all tissues of the parts apical, median and basal, (Fig. 2d) the reaction is weak in the vascular bundle of the apical part of the young rachides.

In the littoral leaflets, the reaction of maüle is much more intense in the adult samples than in the young ones. (Table 4).

The syringyls lignins are abundant in all lignified tissues of young and adult rachides (Fig. 2d) and leaflets (Fig. 2f).

Biometric study

Morphology of fibres: The fibres dissociated and coloured with safranin reveals the presence of a morphological variability. They present unravel points ends (Fig. 3a-c), or festooned (Fig. 3d) slightly (Fig. 3e), or even slightly round. The walls are thick, some ones have round ends (Fig. 3f and h) and can present punctuations (Fig. 3e).

Statistical analysis: The average values of the length expressed in mm, the standard deviation and the variance

Table 4: Description of syringyls lignins in the young and adult leaflets of *Chamaerops humilis* L. of the two sampling regions

		Bs							
		E	H	AxF	iBs	oBs	Ph	X	Me
Young leaflets	T	-	-	+	+	+	-	+	-
	OL	-	-	+	+	+	-	+	-
Adult leaflets	T	-	-	+	++	++	-	+	-
	OL	-	-	+	+	++	-	+	-

T: Tlemcen; OL: Oranian littoral E: Epdemis; H: Hypoderme; AxF: Abaxial fibre; Me: Mesophyla; Bs: Bundle-sheath; iBs: Inner Bundle-sheath; oBs: Outer Bundle-sheath; Ph: Phloem; X: Xylem; (+): Positive reaction; (-): Negative reaction; (++) Contrast

Table 5: The Averages, Standard Deviation and Variances of Fibres of the Young, Adult Rachides and Leaflets of *Chamaerops Humilis* L. of the Two Sampling Regions

Localities		Average	Standard deviation	Variance
YR	T	0.46	0.31	0.1
	OL	0.77	1.10	1.22
AR	T	0.55	0.56	0.26
	OL	1.28	0.70	0.50
YL	T	0.63	0.66	0.37
	OL	1.18	1.06	1.13
AL	T	0.66	0.93	0.88
	OL	1.29	1.07	1.15

T: Tlemcen; OL: Oranian Littoral; YR: Young Rachides; AR: Adult Rachides; YL: Young leaflets; AL: Adult leaflets

Table 6: Values of T calculated for fibres of the young and adult (rachides and leaflets) of *Chamaerops humilis* L. of Tlemcen and of Oranian littoral

Localities	Localities	Young rachides	Young leaflets
AR	T	1.8	
	OL	0.37	
AL	T		0.17
	OL		2.21

T: Tlemcen; OL: Oranian Littoral; AR: Adult Rachides; AL: Adult leaflets

Table 7: Values of T calculated for fibres of the young and adult (rachides and leaflets) of *Chamaerops humilis* L. of the two regions

		Tlemcen			
		Young rachides	Adult rachides	Young leaflets	Adult leaflets
Oranian littoral	Young rachides	3.67			
	Adult rachides		2.21		
	Young leaflets			3.13	
	Adult leaflets				1.97

of 30 fibres measured in the leaflets, young and adult rachides of *Chamaerops humilis* L. of the two sampling regions are presented in Table 5.

$T_1 = 1.8$	$T_1 < T$ threshold
$T_2 = 0.17$	$T_2 < T$ threshold
$T_3 = 0.37$	$T_3 < T$ threshold
$T_4 = 2.21$	$T_4 > T$ threshold
$T_5 = 3.67$	$T_5 > T$ threshold
$T_6 = 2.21$	$T_6 > T$ threshold
$T_7 = 3.13$	$T_7 > T$ threshold
$T_8 = 1.97$	$T_8 < T$ threshold

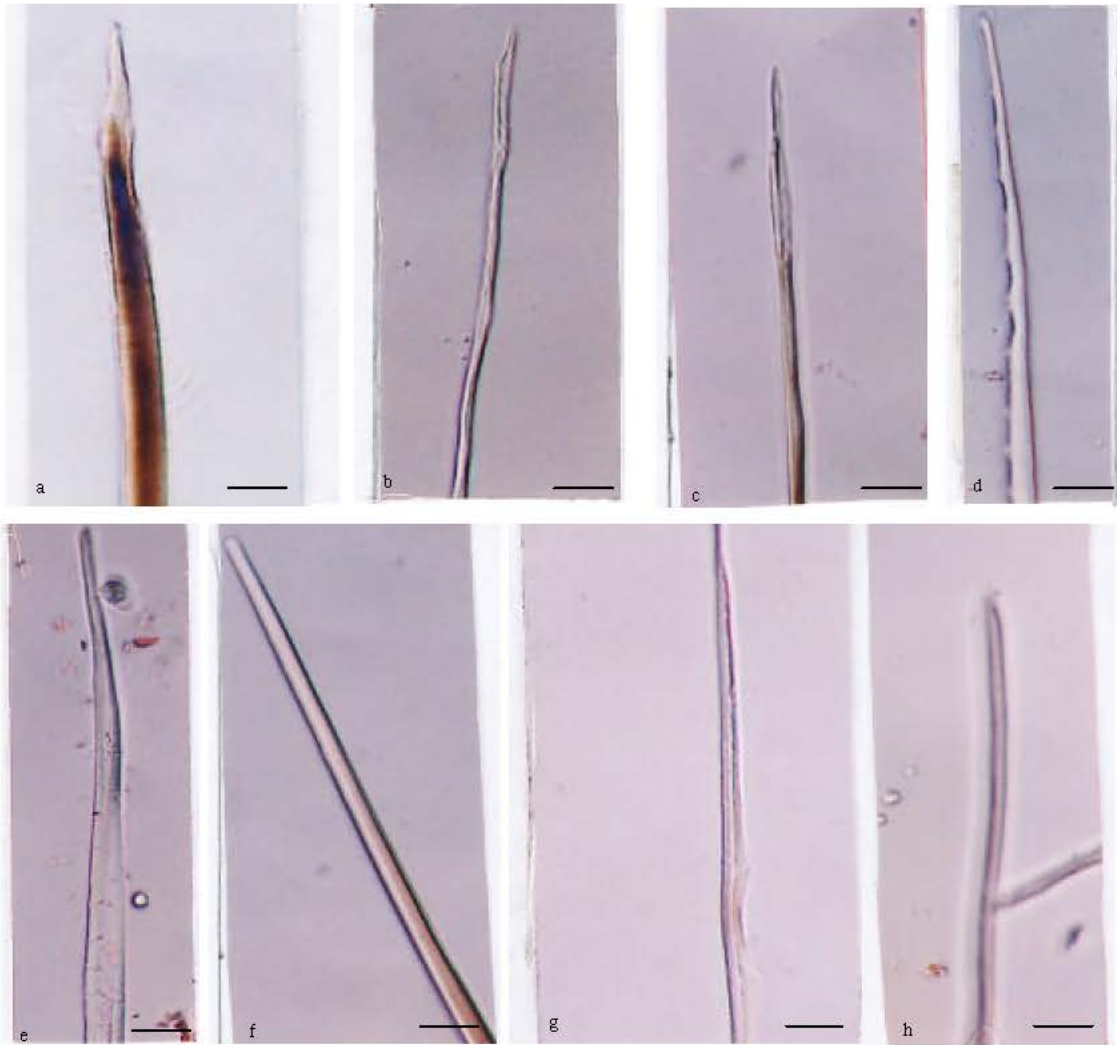


Fig. 3: (a) Morphology of a fibre with unravel point ends X 2100, (b) Morphology of a fibre in bevel end. X 2100, (c) Morphology of a fibre at end at a peak. X 2100, (d) Morphology of a fibre with festooned walls. X 2100, (e) Morphology of a fibre at slightly round end which presents bulk-headings. X 2100, (f) Morphology of a fibre at round end. X 2100, (g) Morphology of a fibre at slightly unravel end. X 2100 and (h) Morphology of a fibre at round end. X 2100. The bar on micrographs represents 5 μ m on Fig. 3a-h

The values of T obtained for fibres of rachides and leaflets compared to T threshold show that there is no difference significant between the average length of young fibres of rachides and adults of the two localities and the young and adult leaflets of the littoral, on the other hand the difference observed is significant for the young and adult leaflets of the littoral (Table 6).

The comparative test lengths of fibres of the samples of the two localities shows that the difference observed between the young and adult rachides and the young leaflets is significant, on the other hand it is not significant for the adult leaflets (Table 7).

DISCUSSION

Our anatomical observations demonstrate that rachides and leaflets of *Chamaerops humilis* L growing in two different habitats points out the structure of the others arecaceae described (Tomnilson, 1961).

This anatomy does not present significant differences however structural variations distinguish the two samples. The palisade parenchyma is well developed in the young and adult limbs of Tlemcen and not very abundant in that of the littoral. This difference is probably

in relation to morphology of the leaflets and with the position of the upper surface of this one compared to illumination.

At the rachides the vascular bundle and the bundle-sheath fibres are developed in the sample of Tlemcen than in that of the littoral, this variation is has to put in relation to the adaptation of the plant to the xerophytic conditions which characterizes this area.

The limbs have vascular bundle of continue «closed type» in taken sample of the oranian littoral and discontinu «open types» in that taken of Tlemcen. This result is an innovation of species of Tlemcen since only the «closed type» is met at the other palm studied in our laboratory, what can be to regards as a significant difference in comparison of the two species.

Also, the fibrous have in the central part of the cellulose cells with wall. These cells are can be at the origin of future vascular bundle (observations already announced at *Washintonia robusta* (Sebaa and Kaid-Harche, 2000).

In parallel, the histochemical data reveal the predominance of syringyls lignins in the rachides and limbs of the two sampling stations, what indeed constitutes the principal originality of the walls of palms and a characteristic of the walls of the angiospermes, low in coniferyls lignins.

The study completed on other palms as *Phoenix canariensis* (Mahi, 2002) and *Washintonia robusta* (Sebaa, 2002) also showed the predominance of the syringyls lignins.

However, biochemical works are to be carried out to determine the value of the syringyls (S) and coniferyls lignines (G) in the *Chamaerops humilis* L.

It was also shown that lignins from adult *chamaerops* are relatively richer in syringyls than the young, suggesting a progressive lignin deposit in fontion of the age.

Also, the lignification starts in the basal parts then continuous towards the parties top, like that observed at other species like *Aristida pungens* Desf. (Harche and *et al.*, 1992).

The biometric study shows that the average length of fibres of the young rachides of *Chamaerops* of Tlemcen is of 0.46 mm and 0.55 mm for the adult rachides.

The statistical study shows that there are no significant differences between the averages of fibres of rachides (T calculated is lower than T threshold).

The test of comparison realized between the young rachides and adult of the two stations shows that there is a significant difference between the averages of its fibres.

This difference suggests that the fibres are not at the same stage of differentiation since at the adult stage this difference is not significant.

For the young leaflets of Tlemcen, the test shows that these fibres have an average length of 0.63 mm and the fibres of the adult palms have an average of 0.66 mm. Its statistical tests not of differences significant between leaflets of Tlemcen where T calculated is definitely lower than T threshold: $0.17 < 2$ (T threshold).

The average lengths of fibres is of 1.28mm for the adult rachides and 0.77 mm for the young rachides of the palms taken in the oranian littoral, this species is a fibrous plant average and present a non significant difference.

The comparative study was also carried out between the bodies of the two stations, the results showed that there is a significant difference between the young and the adult rachides and adult palms of the two stations enter they, these results make it possible to suggest that these species probably come from two different populations and that can be one is more advanced than the other. Studies cytogenetics are in hand to check this assumption.

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