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COMPARATIVE ANATOMICAL STUDIES ON LEAVES AND STEM OF CERTAIN TAXA OF CONIFERS AND THEIR SYSTEMATIC SIGNIFICANCE

Fouda, R. A.
Agric. Botany Dept., Fac. of Agric., Mansoura Univ., Mansoura, Egypt.

ABSTRACT

Eight conifer taxa belonging to four families, *i.e.*, *Pinaceae*, *Taxodiaceae*, *Araucariaceae* and *Cupressaceae* were collected during spring and summer of 2003 season to compare the anatomical structure of both leaves and stems and evaluate their systematic significance. The obtained results could be summarized as follows:

Regarding the leaf structure, stomata type and their position, presence or absence of the hypodermis and endodermis; number of resin ducts, differentiation of mesophyll tissue and forms of transfusion tissue were varied within the studied taxa. *Taxodium distichum* leaves are characterized with differentiation of mesophyll tissue and spongy parenchyma, while, in the other studied taxa there is no such distinction. Needles of *Pinus* species are characterized with largest number of resin ducts, presence of the endodermis and infloding parenchyma cells in the mesophyll tissue compared with the other studied taxa.

.Concerning the stem structure, the number and arrangement of resin ducts, number and thickness of eipthelial cell layers varied among the studied taxa. There are two types of resin ducts in *Pinus* species, *.i.e.*, primary and secondary which are found in cortex and xylem tissue, respectively, while in other examined taxa were found only primary resin ducts. The largest number of resin ducts with scattered arrangement was found in *Araucaria*. The largest diameter of resin ducts with more number of epithelial cell layers was found *Juniperus communis*, while, the smallest diameter of resin ducts was found in *Thuja*. These characters may be used as a guide for classification of conifer families.

The obtained results indicate also similarity of *Taxodium* with the *Cupressaceae* family in certain anatomical leaf and stem characters, *i.e.*, absence of hypodermis and endodermis, number of resin ducts. Its pattern and structure in the leaf, as well as number and arrangement of resin ducts and its position in the stem.

INTRODUCTION

Conifers include certain families, which having ornamentals, agricultural and medicinal importance. Conifers are the main source of various types of resins, essential oils, tannin and wood (Vashishta, 1983).

Attempts were carried out to classify the conifers based on cone structure, embryological, palynological anatomical features (Hart, 1987). He pointed out that the anatomical features could be used for recognizing relationships among conifers. Chaturvedi (1993) used leaf anatomy, epidermal features and stomatal frequency as well as number of resin canals to distinguish between three species of *Taxodiaceae*.

In conifers, resin ducts are a common structure of the plant body. It may present in primary and secondary tissues (Fahn, 1990). He added that the secondary resin ducts in conifers are important in wood technology and resin extraction. The resin ducts are an important diagnostic character in

taxonomy of *Pinus* species (Duffield, 1991) and particularly on other conifers. He described four types of resin ducts in the mesophyll tissue of pinus needles according to their position, i.e., external, medial, endonal and spetal. Furthermore, the number, arrangement and structure of resin ducts in the mesophyll tissue as well as type and stomata on the leaf structure may have a significant taxonomic value per identification of conifer families.

On the other hand, Eckenwalder (1976) noted that Cupressaceae and Taxodiaceae are usually distinguished only by leaf form and phyllotaxis. He proposed that the families should be marged.

The present study aimed to compare the anatomical structure of both leaves and stems of certain conifer taxa referring to resin ducts characteristics such as number, position, types, arrangement and their structure. Another objective was the possibility of establishment a taxonomic relationship between the investigated taxa based on the anatomical studied characters.

MATERIALS AND METHODS

Comparative anatomical studies of leaves and stems between certain taxa of conifers and systematic significance were investigated.

For standardization, of the anatomical characters, samples were taken from uniform one-year-old shoots of eight different coniferous taxa belonging to four families, *i.e.*, *Pinaceae*, *Taxodiaceae*, *Araucariaceae* and *Cupressaceae*. Fresh samples were collected during spring and summer of 2003 from the gardens of Mansoura University and different locations from Mansoura region. The investigated taxa are, *Pinus haleponsis* Mill; *Pinus canariensis* Chr. Sm., *Taxodium distichum* (L.) Rich., *Araucaria heterophylla* (Salisb.) Franco, *Cupressus sempervirens* L., *Cupressus macrocarpa* Martw., *Juniperus communis* L. and *Thuja crientalis* L.

1- During collection of samples, certain morphological characters, *i.e.*, leaf form and phyllotaxy were recorded.
2- For the anatomical investigation of leaves, samples (0.5 cm) were taken from the middle region of the needles, which occurred at the middle part of one old shoots.
3- For studying the anatomical structure of stem, samples (0.5 cm) were taken from the middle part of one year old shoots.

Cross sections at 15-20 µm thick were made using a rotary microtome after proceeding the usual paraffin methods (Gerlach, 1977). Sections were stained in saffranin-light green combination, cleared in clove oil and mounted in canada balsam.

Sections (mean of 10 sections) were examined microscopically and the following characters were recorded:

1- Leaf sections
- Types and position of stomata.
- Presence of mesophyll tissue.
- Presence of hypodermis and endodermis.
- Resin ducts number, position, diameter and structure.

- Number of vascular bundles.

2- Stem sections

- Number of resin ducts, their arrangement and diameter as well as number of epithelial cell layers for each duct.

RESULTS AND DISCUSSION

1- Morphological observations:

The most remarkable morphological features in all examined coniferous taxa are leaf forms and phyllotaxy (Table 1). The three different leaf forms in the examined conifers were observed as follows:

1. The needle-like leaves, was found in *Pinus* species.
2. The leaves are linear as in *Taxodium* and *Araucaria*,
3. The scale-like leaves were found in *Cupressus* species.

The phyllotaxy of all examined conifers is spiral. *Cupressaceae* characterized by an opposite arrangement. The above mentioned two morphological characters are important for conifers classification (Vasishta, 1983). In addition, Eckenwalder (1976) noted *Cupressaceae* and *Taxodiaceae* are usually distinguished only by leaf form and phyllotaxis.

Table (1): some morphological features in studied taxa of conifers.

Families	Taxa	Leaf form	Phyllotaxy
Pinaceae	*Pinus hedepensis* Mill	Needle-like leaves	Spiral
	Pinus canariensis Chr. Sm		
Taxodiaceae	*Taxodium distichum (L.)*	Linear	Spiral
Araucariaceae	*Araucaria heterophylla* (Salish)	Linear	Spiral
Cupressaceae	*Cupressus sempervirens* (L.)	*Scale*-like	Opposite
	Cupressus macrocarpa Martw.		
	Juniperus communis (L.)		
	Thuja orientalis (L.)		

2- Comparative leaf structure:

Data presented in Tables (2) and illustrated in Figs. (1-8) indicate that shape of transverse sections are half circle and triangle in Pinaceae; Square in Araucaria; Flattened in Taxodium, while, in *Cupressaceae* species are conical and ovat-rectangular.

A- Epidermis:

It forms the outermost cellular layer on both surfaces of the leaf, the epidermal cells are thick-walled and covered with thick cuticle. It serves to check excessive transpiration.

B- Stomata:

Data in Table (2) and illustrated in Fig. (1) show that stomata are present on all surfaces or found only on the adaxial or abaxial epidermis, Sunken in *Pinaceae* (Fig. 9) and the other type is hyperstomata in *Taxodiaceae* and *Cupressaceae*. The stomata varied in their distribution and arrangement according to the family. In *Pinaceae* and *Araucariaceae*, the stomata are found on all sides of the leaves (Fig. 1). This arrangement is called amphistomata (Vasishta, 1983). In *Cupressaceae* stomata present

Table (2): Certain anatomical characters of the leaves in studied coniferous taxa.

Taxa	T.S. shape	Position of stomata	No. of hypodermis cell layer	Presence of endodermis	Differentiation of mesophyll tissue	Resin ducts				No. of epithelial cell layers	No. of vascular bundle
						Number	Position	Pattern	Dimension um		
Pinus hedepensis	Half-circle	All sides of the leaves	2	+	-	6	External	Circle	104	2	2
Pinus canariensis	Triongle	All sides of the leaves	4	+	-	7	External	Circle	82	1	2
Taxodium distichum	Flattened	Abaxial epidermis	-	-	+	1	Between the vascular bundle and abaxial epidermis	Circle	88	1	1
Araucaria heterophylla	Squair	All sides of the leaves	2	-	-	7	External	Circle	74	1	3
Cupressus sempervirens	Conical	Adaxial epidermis	2	-	-	1	Under the adaxial epidermis	Circle	169	2-3	1
Cupressus macrocarpa	Rectangle	Adaxial epidermis	-	-	-	1	Under the adaxial epidermis	Circle	98	3	1
Juniperus communis	Conical	Adaxial epidermis	-	-	-	1	Under the adaxial epidermis	Circle	306	5	1
Thuja orientalis	Conical	Adaxial epidermis	2	-	-	1	Under the adaxial epidermis	Circle	178	1	1

+ : Presence.
-: Absence.

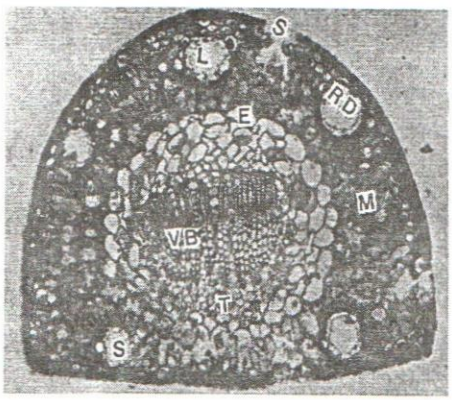

Fig. (1): Needle cross section of *Pinus halepensis* showing sunken stomata (S) are found in all sides of the leaf, presence of endodermis and two types of resin ducts (RD), large (L) and small (S) (O.C.X10. Obj. X 10).

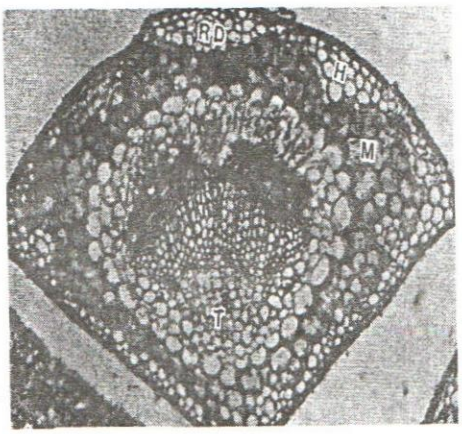

Fig. (2): Needle cross section of *Pinus canariensis* . Note the hypodermis occurs in distinct patches (O.C.X10. Obj. X 10). M: Mesophyll tissue, E: Endodermis, T: Transfusion tissue, VB: Vascular bundle, RD: resin duct.

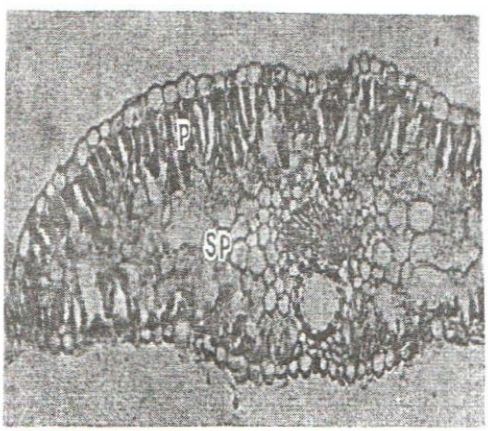

Fig. (3): Leaf cross section of *Taxodium distichum* showing a well-developed palisade (P) and spongy parenchyma (SP) (O.C.X10. Obj. X 10).

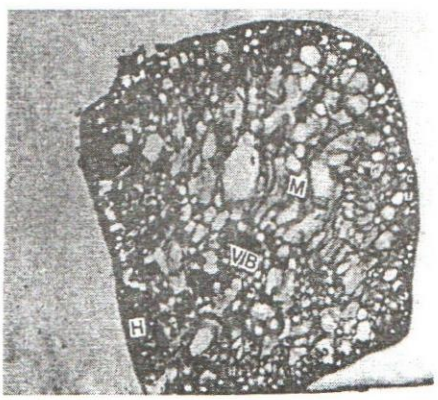

Fig. (4): Cross section of *Aracaria heterophylla* the hypodermis (H) occurs in distinct patches, three vascular bundle (VB) and no differentiation of mesophyll tissue (M) (O.C.X10. Obj. X 10).

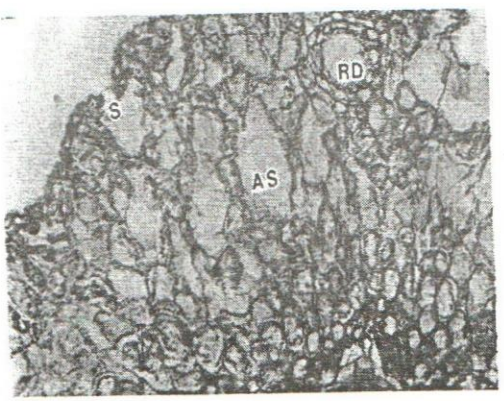

Fig. (5): Cross section of *Cupressus sempervirens* scale-like leaf. Note a well developed air space (AS) in the mesophyll tissue and the stomata (S) are found only on the adaxial surface (O.C.X10. Obj. X 10).

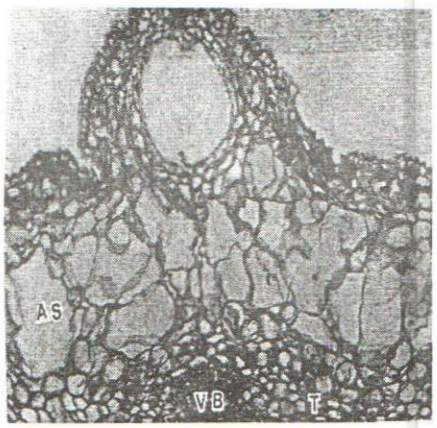

Fig. (6): Cross section of *Juniperus communis* scale like leaf showing the transfusion tissue (T) forms two lateral that arise from the sides of vascular bundle (VB) (O.C.X10. Obj. X 10).. AS: AS, Air space.

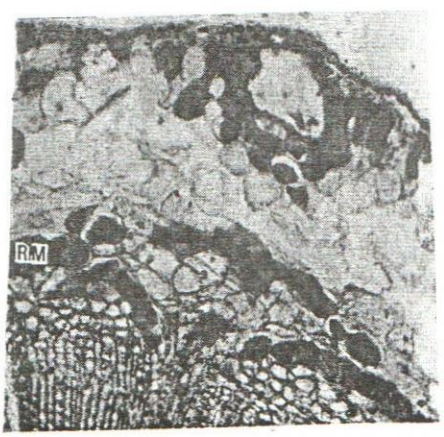

Fig. (7): Cross section of *Cupressus macrocarpa* Hartw scale-like leaf showing more diposition of resin materials (RM) in the mesophyll tissue (O.C.X10. Obj. X 10).

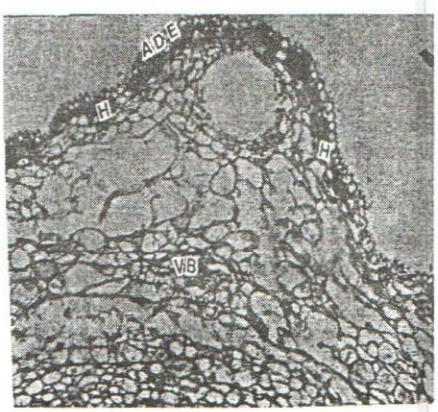

Fig. (8): Cross section of *Thuja orientalis* scale-like leaf showing the hypodermis (H) present beneath the adaxial epidermis (AD.E) and occurs one resin duct and vascular bundle (VB) (O.C.X10. Obj. X 10).\

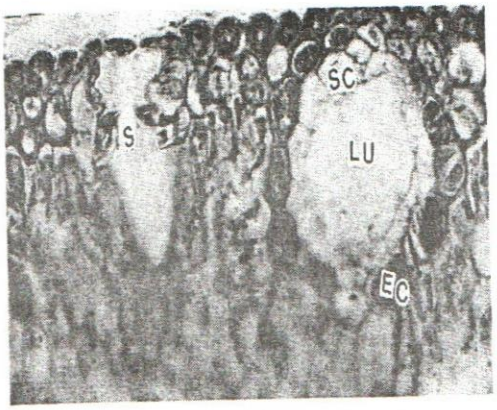

Fig. (9): Portion of cross section in *Pinus halepensis* leaf showing sunken stomata and external resin duct (O.C.X10. Obj. X 10). S: Stomata; RD: Resin duct. EC: Epthelial cells ; Lu: Lumon; Sc: Sheath cells.

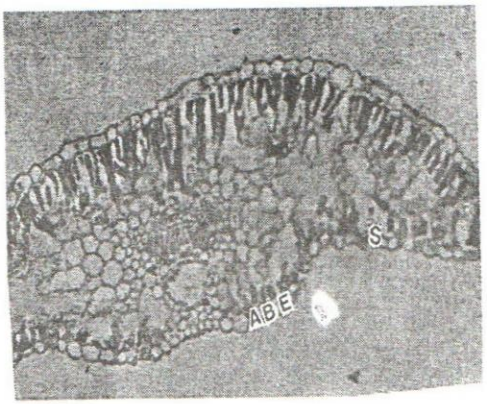

Fig. (10): Cross section in *Taxodium distichum* leaf showing the stomata (S) are found only on the adaxial surface (AB. E) (O.C.X10. Obj. X 10).

only on the adaxial surface (Fig. 5) and called hyperstomatic, whereas, in Taxodium, are found only on the abaxial epidermis (Fig. 10).

C- Hypodermis:

It forms a single or more layers of thick end wall cells under beneath the epidermis. The hypodermis cell layers varied in number, position and their arrangement in the studied taxa. In *Pinus* species they are under beneath the epidermis and ranged between 1 and 5 cell layers. It is also noted in *Pinus canariensis* that it is found under beneath all sides of the needles and occurs in distinct patches (Fig. 2). In *Araucaria*, it occurs in distinct patches that are well developed at the corners. The hypodermis is absent in the leaves of *Taxodiaceae* and *Cupressaceae*, whereas, in *Thuja* it is present beneath the adaxial epidermis (Fig. 8).

D- Mesophyll tissue:

Data in Table (2) and Figs (3 and 4) indicate that there are two types of leaves in all examined taxa. In *Taxodium*, there is a well-developed palisade tissue and spongy parenchyma. The palisade tissue seems to be obvious under the adaxial epidermis and less found beneath the abaxial epidermis. In the other studied taxa there is no such distinction (Fig. 4). In *Pinus* species the cells of mesophyll show infoldings (Fig. 1), while cupressus species show well-developed air space (Fig. 5). Fahn (1990) described two types of gemnosperm leaves depending on differentiation of mesophyll tissue.

E. Resin ducts:

Resin ducts appear as a normal feature of the conifer leaves. They found in the mesophyll tissue. Sheue *et al.* (2003) observed two forms of resin ducts in needles of *Pinus taiwanensis*, primary and secondary. Primary ducts were found in the mesophyll tissue, and secondary ducts were found within the vascular tissue. Resin ducts in all studied taxa are found in the mesophyll tissue of the leaves and develop schizogenously. Each duct consists of lumen, which surrounded by two kinds of cells (Fig. 9). The intermost layer is called sheath cells. These cells enclosed by one or more cell layers which called epithelial cells (Fahn, 1990).

Data in Table (2) indicate that the resin ducts have a great diversity in number, position, structure and their dimension depending on the taxa. It is clear that shape of resin ducts in all examined leaves is circle. Data in Table (2) show that the number of resin ducts varied greatly among the different studied taxa. It ranged from one in *Taxodium* and *Cupressus* species to 7 in *Pinus* and *Araucaria*. Furthermore, there are two types of resin ducts found in the mesophyll tissue of *Pinus* needles, *i.e.*, large and small (Fig. 1).

Regarding position of resin ducts in the leaves. Resin ducts in the *Pinaceae*, *Araucariaceae* and *Cupressaceae* taxa were called external, which in contact with the hypodermis (Fig. 9), whereas in *Taxodiaceae*, there is one resin duct between the vascular bundle and abaxial epidermis (Fig. 3). In *Cupressaceae* taxa there is also one resin duct located under the adaxial epidermis (Figs. 5 and 6).

The diameters of resin ducts varied also between the studied taxa. It is clear that, the largest average diameter of resin duct (including lumen, sheath and epithelial cells) was found in *Juniperus communis* L. (Fig. 6) and the lowest one was found in *Araucaria heterophylla* (Salisb) Franco. (Fig. 4). Sheue *et al.* (2000) compared the variation of 28 characters of *Pinus* needle structures, mainly the variation of resin duct number and their area. Sheue *et al.* (2003) compared the variation of resin ducts including number position, type, pattern and their size. They noted that epithelial cells of ducts were the main source of resin secretion and the resin ducts in the mesophyll are useful for *Pinus* species identification.

Transfusion tissue is characteristic of the conifer leaves. In Pinaceae it is enclosed by endodermis (Fig. 1). In *Cupressaceae* this tissue forms two lateral wings that arise from the sides of the vascular bundle (Fig. 6). In *Taxodium* (Fig. 3) and *Araucaria*, transfusion parenchyma cells surround the vascular bundles. Similarly, Esau (1977) reported that endodermis is the boundary between the vascular region and the mesophyll. She added that it is not equal clear in conifer leaves only the *Pinaceae*. The number of vascular bundles varied also among taxa. It is double in the two examined *Pinus* species and single in both *Taxodium* and *Cupressaceae* (Figs. 3 and 8), but in *Araucaria* they are three (Fig. 4).

From the above mentioned results, it could be concluded that stomata type and their position; present or absent, the hypodermis and endodermis; number, diameter, position and structure of resin ducts especially the number of epithelial cell layers, differentiation of mesophyll tissue and forms of transfusion tissue were varied within the studied conifer

members and these characters may be used as a guide for classification of conifer families.

3. Comparative stem structure:

The stem structure of conifers stem equals in general description of gymnosperm stem described by Fahn (1990). The stem having collateral and open vascular bundles, which arranged in a complete cylinder (Fig. 12).

The most remarkable anatomical features of conifer stems are early formation of secondary vascular tissues and periderm tissue (Fig. 11).

Data in Table (3) and (Figs. 11, 12, 13 and 14) revealed that the number of resin ducts varied among the different studied members. The largest average number of resin ducts was found in *Aruacaria heterophylla* (Fig. 12). The next largest average number of resin ducts was found in *Pinus* species, and the fewest number was found in *Cupressus* species. Among of them *Cupressus macrocarpa* and *Thuja orientalis* are leaky resin ducts (Table 3).

Concerning, the arrangement of resin ducts, data in the same table indicate that the arrangement of resin ducts also varied among taxa. It is clear that the arrangement of resin ducts in the stem of all examined taxa are circle, except, scattered arrangement in *Araucaria heterophylla*. Esau (1977) reported that the arrangement and number of resin ducts are variable even in the same species.

Data in the same Table indicate also that, in *Pinus* species, the resin ducts are found in both cortex and xylem tissues (Fig. 11), while in other examined taxa, they found only in the cortex tissue. Fahn (1990) noted that two types of resin ducts were found in *Pinus halepensis* according to its position, *i.e.*, primary ducts are found in the cortex and secondary are found in the xylem tissue. He added that the secondary ducts are important in wood technology and resin extraction.

Comparing the average dimension of resin ducts in different examined coniferous taxa, it is noted that two types of resin ducts were found, depending on their diameter, large and small. It is clear that the largest average dimension of resin ducts was found in *Juniperus communis* (Fig. 15) while, the lowest was found in *Thuja orientals* (Fig. 17). In certain taxa, the largest resin duct might be two times the dimension of the smallest one Table (3).

Regarding, the epithelial cells, data in Table (3) revealed that, the number and thickness of epithelial cell layers varied among different taxa (Figs. 15, 16 and 17). The largest number and thicker epithelial cell layers were found in *Juniperus communis* (Fig. 15). Epithelial cells of ducts play an important role in resin secretion (Sheue *et al.*, 2003). These cells secrete and contain polyphenolic materials, which play an important role in plant defense against a range of herbivorous organisms and pathogen (Franceschi *et al.*, 1998).

From the above mentioned results, it could be concluded that, the number and arrangement of resin ducts, its position as well as diameter of resin ducts, number and thickness of epithelial cell layers varied among the

Table (3): Certain anatomical characters of the leaves in studied coniferous taxa.

Taxa	No. of resin ducts	Arrangement of resin ducts	Position of resin ducts		Type of resin ducts		Resin ducts dimension (µm)		Epithelial cell layers	
			Cortex	Xylem	Large	Small	Large	Small	No.	Thickness
Pinus halepensis	15	Circle	+	+	11	4	220	136	1	40
Pinus canariensis	16	Circle	+	+	14	2	220	140	1	32
Taxodium distichum	6	Circle	+	-	3	3	212	130	1	28
Araucaria heterophylla	27	Scattered	+	-	8	19	208	112	1	36
Cupressus sempervirens	7	Circle	+	-	2	-	128	-	3	32
Cupressus macrocarpa	2	Circle	+	-	2	-	100	-	1	28
Juniperus communis	9	Circle	+	-	5	-	270	140	5	80
Thuja orientalis	2		+	-	2	-	98	-	1	36

+ : Presence.
-: Absence.

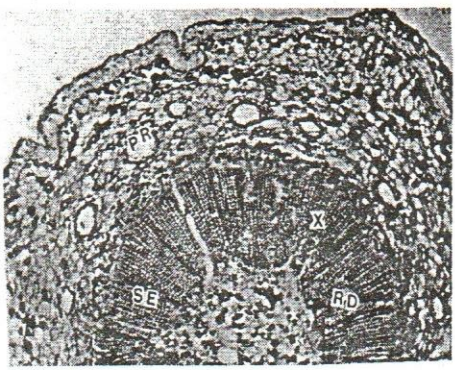

Fig. (11): Cross section of *Pinus halepensis* stem showing two types of resin ducts, primary (P) are found in the cortex and secondary (SE) in the xylem tissue (O.C.X10. Obj. X 10).

Fig. (12): Cross section in *Araucaria heterophylla* stem showing large number of scattered resin ducts (RD) (O.C.X10. Obj. X 10).

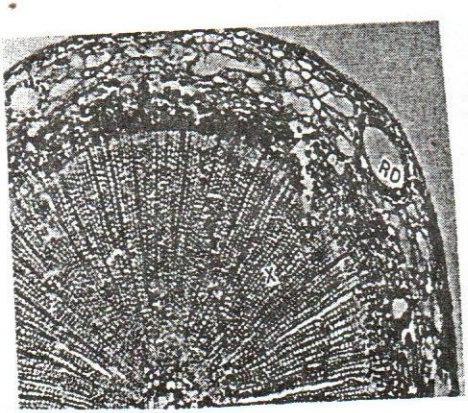

Fig. (13): Cross section of *Taxodium distichum* (L.) stem. Note primary resin ducts (PR.) only and early formation of secondary vascular tissues (O.C.X10. Obj. X 10). X, xylem R, Resin duct.

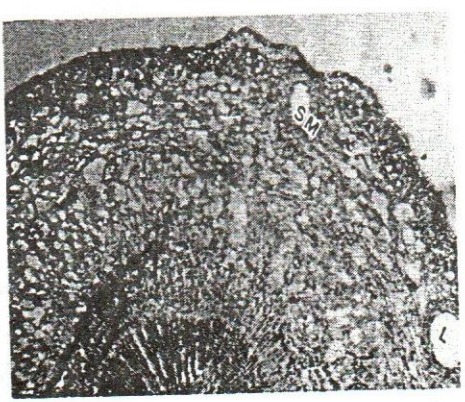

Fig. (14): Cross section of *Juniperus communis* stem showing two types of primary resin ducts large (L) and small (Sm) (O.C.X10. Obj. X 10).

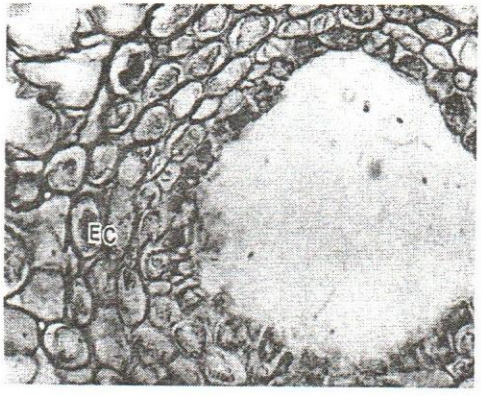

Fig. (15): Resin duct in the stem of *Juniperus communis* L. showing largest diameter of resin duct with largest number of epithelial cell layers (EC) (O.C.X10. Obj. X 10).

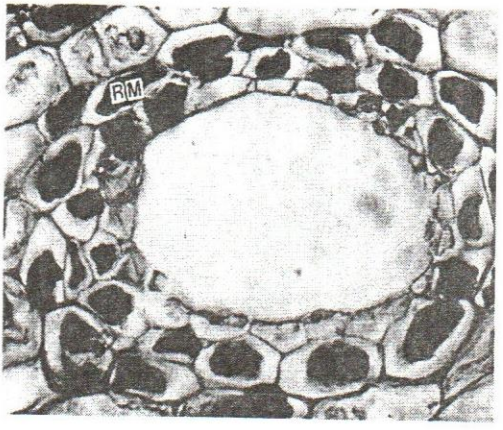

Fig. (16): Resin duct in the stem of *Taxodium distichum*. showing two epithelial cell layers (EC) and more diposition of resin materials within it. (O.C.X10. Obj. X 10).

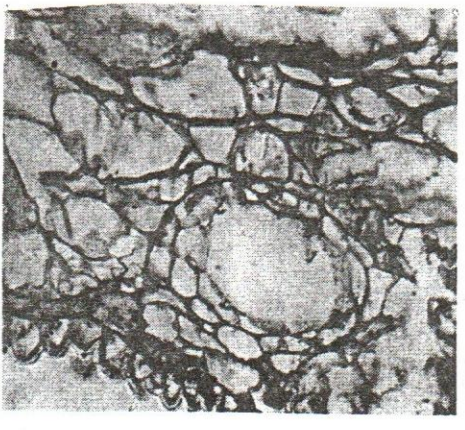

Fig. (17): Resin duct in the stem of *Thuja orientalis* L. showing the smallest resin duct (RD) (O.C.X10. Obj. X 10).

studied coniferous taxa. These characters may be useful for conifer families identification.

The obtained results indicate also, similarity of *Taxodium* with the *Cupressaceae* family in certain anatomical leaf and stem characters, *i.e.*, hypodermis and endodermis development, number of resin ducts in the leaf, its pattern and structure especially epithelial cell layers, in addition to number and arrangement of resin ducts in the stem and its position. Therefore, the results agree with those obtained by Eckenwalder (1976) and Price and Lowenstein (1989). They concluded that, both *Taxodiaceae* and *Cupressaceae* should be merged and treated as a single family.

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دراسة مقارنة للتركيب التشريحى للورقة والساق لبعض الفئات التصنيفية للمخروطيات وأهميتها التقسيمية

رمضان عبد المنعم فودة

قسم النبات الزراعى – كلية الزراعة – جامعة المنصورة – المنصورة – مصر

نظرا لأهمية المخروطيات فى إفراز وإنتاج المواد الراتنجية التى تلعب دورا أساسيا فى النظام الدفاعى لهذه النباتات ضد الكثير من الكائنات الحية الدقيقة الممرضة فقد تم تجميع نباتات من ٨ فئات تصنيفية من المخروطيات الموجودة بمدينة المنصورة تنتمى إلى أربع عائلات هى الصنوبرية والتاكسودية والأروكارية والسروية خلال فصلى الربيع والصيف عام ٢٠٠٣ لإجراء دراسة مقارنة للتركيب التشريحى لكل من الورقة والساق فى تلك الفئات التصنيفية وكذلك لأهميتها التقسيمية .

ويمكن تلخيص النتائج المتحصل عليها فى الآتى :

بالنسبة للتركيب التشريحى للورقة ، إختلفت الوحدات التصنيفية موضع الدراسة فيما بينها فى نوع الثغور وتوزيعها ، ووجود أو غياب تحت البشرة والاندودرمس ، وعدد القنوات الراتنجية ، وتميز النسيج المتوسط وشكل النسيج الناقل . وقد تميز النسيج المتوسط فى ورقة نبات التاكسوديم إلى النسيج العمادى والإسفنجى بينما لم يظهر مثل هذا التميز فى الفئات التصنيفية الأخرى . كما تميزت أوراق الصنوبر بوجود أكبر عدد من القنوات الراتنجية ، إضافة إلى وجود الاندودرمس وكذلك البارنكيما ذات الجدر المطوية فى النسيج المتوسط دون غيرها من الفئات التصنيفية المدروسة .

أما بالنسبة للتركيب التشريحى للساق ، فقد وجد أن عدد وترتيب الغدد الراتنجية ومكان وجودها وكذا أبعادها ، وعدد وسمك الخلايا الطلائية المفرزة إختلفت فيما بين الفئات التصنيفية المدروسة . وقد وجد فى سيقان أنواع الصنوبر نوعان من الغدد الراتنجية ، إبتدائية فى نسيج القشرة ، وثانوية فى نسيج الخشب ، فى حين وجد غدد إبتدائية فقط فى باقى الفئات التصنيفية المدروسة . كما أوضحت النتائج أن ساق شجرة عيد الميلاد يحتوى على أكبر عدد من القنوات الراتنجية مبعثرة فى القشرة . وقد تميز جنس العرعر بوجد أكبر الغدد حجما مع وجود عدد أكبر من طبقات الخلايا الطلائية بينما وجدت الغدد الأقل حجما فى جنس التويا ، ومثل هذه الصفات قد تستخدم كمفتاح لتقسيم العائلات المخروطية .

أكدت النتائج أيضا تشابه جنس التاكسوديم مع العائلة السروية فى بعض الصفات التشريحية للورقة والساق مثل غياب تحت البشرة والأندودرمس ، وعدد وشكل القنوات الراتنجية فى الورقة بالإضافة إلى عدد وترتيب ومكان الغدد الراتنجية فى الساق .