# Comparative Studies on Structural Feature of Vessels of Four Genera of Cycads with Angiosperm*

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**Abstract**: The tissues of leaf of *Cycas taiwaniana*, *Bowenia serrulata* and *Dioon edule* and tissue of root of *Macrozamia longispina* of cycads, and leaves of *Michelia alba* and *Amygdalus persica* of angiosperm were observed by scanning electron microscope. The results showed that there are many types of vessels in four genera of two families of cycads, the manner and process of development and constitution in structural characteristics of vessel of cycads and angiosperm are identical, some characters of vessel such as inclined extent of perforation plate of end wall etc. showed that some angiosperm are more primitive then that of cycads. In vessels of species of angiosperm, many are band shape, and without end wall, only have two lateral walls and another two narrow margins, the top is acute or with an arc margin, many perforations located in lateral wall. Cognized to vessel of cycads, help us to understand the mechanism which these most primitive seed plants extant how to adapted to harsh environments and their evolutionary level, and has significance to use efficiency measure protect these rarity plants and has significance to plant anatomy, plant systematics and plant evolution.

**Key words**: Cycads; vessel; angiosperm; evolution; structural characteristic; adaptability
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Cycads are very primitive seed plants of present existence, they still possess primitive characteristics in external morphology and internal structure, such as sperm with flagellum. They are classified into three families: Cycadaceae, Stangeriaceae and Zamiaceae. In the past, it was believed that the water-transporting elements in cycad vascular tissue would be only primitive tracheids[1-6]. Huang and Zhang [7] published a brief report that first discovered vessel in *Zamia furfuracea* of cycads, Lin &. Huang [8] published another brief report on vessel feature in root of *Cycas elonga*, Huang and Wu [9] discovered vessel in *Cycas panzhihuaensis*.

Further research into the structural characteristics of their tissues is important for taking effective measures to cultivate, breed and create the right circumstances to enlarge the population and the natural community. The present study makes a comparative research on cycads of four genera with angiosperm in the structure feature of the xylem of the vascular tissue is an attempt to address this deficit.

## 1 Materials and methods

The pinnas of leaf of *Cycas taiwaniana* Carruth. of Cycadaceae, *Dioon edule* Lindl. of *Dioon* genus, root of *Macrozamia longispina* P. I. Forst. & D. L. Jones of Zamiaceae were collected from living cycads in the Cycad Garden of Shenzhen Fairy Lake Botanical Garden, Shenzhen, China. The pinna of leaf *Bowenia serrulata* (W. Bull) Chamb. of Zamiaceae is collected from South China Botanical Garden, Guangzhou, China, leaves of *Michelia alba* DC. Syst. and *Amygdalus persica* Linn. were collected from the campus of Guangxi University, Nanning, China. The voucher specimens: *Cycas taiwaniana* pinna: Y. Y. Huang & Y. H. Wu 376, *Bowenia* serrulata pinna: Y. Y. Huang & L. J. Wei 361; *Dioon edule* pinna: Y. Y. Huang & A. H. Huang 384, *Macrozamia longispina* root: Y. Y. Huang & Y. H. Wu 388, *Michelia alba* leaf: J. Z. Wang & Y. Y. Huang 020, *Amygdalus persica* leaf: J. Z. Wang & Y. Y. Huang 019, are deposited at the Herbarium of the Agricultural College of Guangxi University (GXAC). After collection, these materials were cut into 1

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cm × 1 cm in size, and fixed immediately in FAA (formalin 40%: acetic acid in glacial: ethanol 70%, 5:5:90 in vol.) macerated and dissociated by Jeffrey's technique [10, 11], and rinsed with water. The dissociated materials were dehydrated in a graded series of ethanol / isoamyl acetate and critical point-dried in $CO_2$, then sputter-coated with gold for observation and photography using XL - 30 ESEM and JEM - 1200Ex/s scanning electron microscope (SEM).

## 2 Results

Examination of dissociated materials of the leaf and root tissues of four cycad genera and two genera of angiosperm showed that there are many vessel elements in the conducting tissue of the xylem. The structural characteristics of vessels in the four cycad plants and two plants of angiosperms are described below.

*Cycas taiwaniana* There are annular, spiral, scalariform vessel (Figure1A, B), pitted vessel and reticular vessels (Figure 1C, D) in the pinna of leaf. The shapes of these vessels are nearly square, polygonal, nearly circular etc. There are both simple and multiple perforation plates in the end wall. The end walls of annular vessel are general simple perforation plate and possess nearly horizontal or horizontal perforation plate, a large perforation locate in perforation plate of end wall (Figure 1A). There are more of the multiple perforation plates, with three to ten perforations in each plate (Figure 1B & D), and their end wall perforation plate are mainly inclination. Some end wall perforation plate have a little tail, and with a twisting shape.

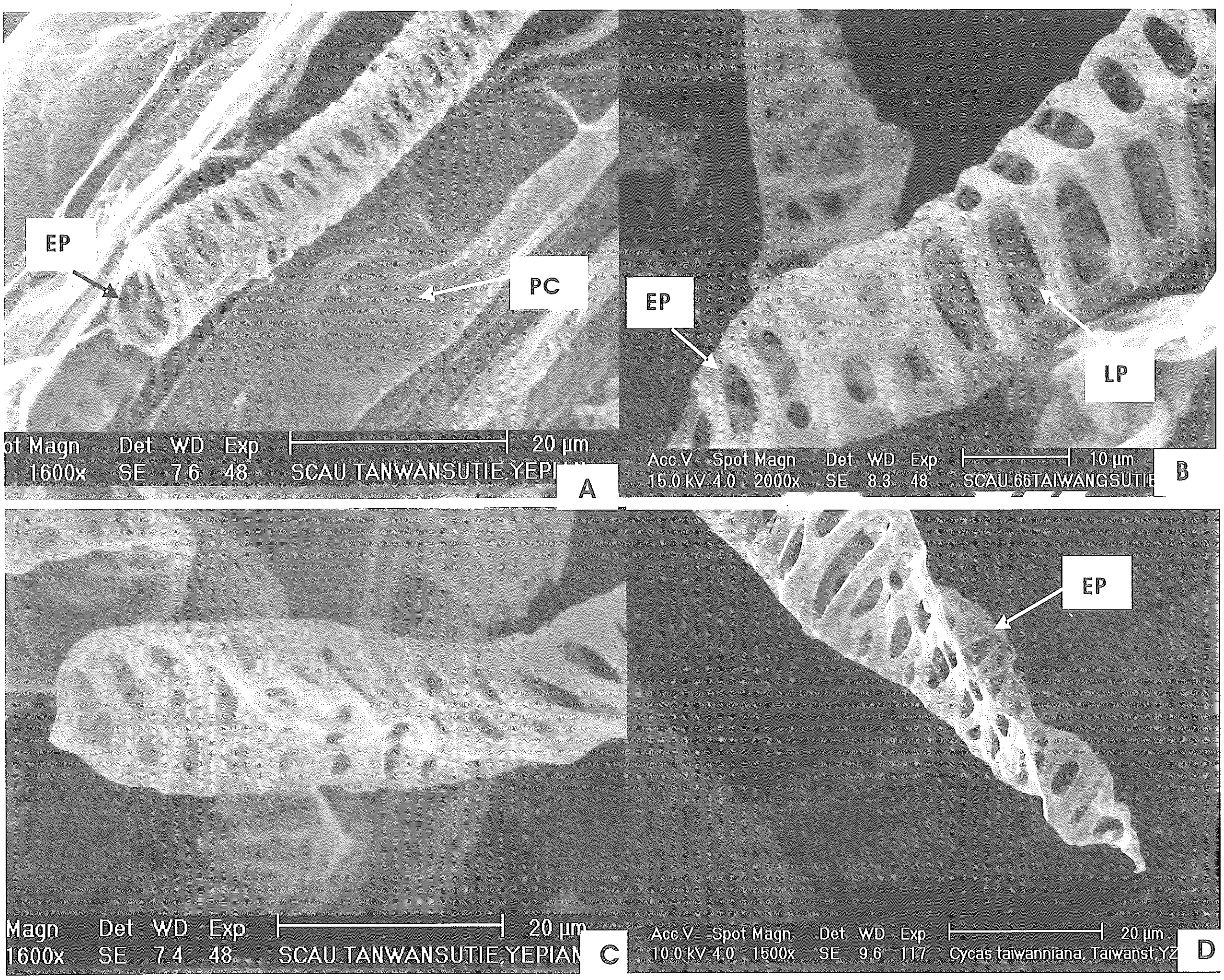


Fig. 1 SEM photographs of vessel elements of leaf of *Cycas taiwaniana*

A: A spiral vessel element which possess a large perforation in horizontal perforation plate. (EP: End wall perforation plate, PC: Parenchyma cell). B: A salariform vessel element with a scaraliform perforation plate in end wall and some lateral wall perforations. (LP: Lateral wall perfration). C: Pitted vessel element, which possess a near horizontal end wall perforation plate with several peforations. D: A reticular vessel element, it has a inclined mutiple perforation plate in end wall, and with a little tail

The portion of lateral walls near the end walls consist of only primary walls without secondary thickening formed also perforations. In some larger perforations, the ornamentation and perforations can be seen located in the interior wall of the vessels (Figure 1B). Analyzed to these perforations showed: their diameter or length are very large, such as in simple perforation plate of end wall of annular vessel, the diameter of a circular perforation have approached 8 μm, the lengdth of perforations located in multiple perforation plates have reached 10 ~ 12 μm, their width can reach 4 ~ 5 μm; compare with many angiosperms which their diameter of perforation of vessel have only 2 μm [12]; or length is only 6 ~ 8 μm, width only is 3 ~ 5 μm [13], illuminated that cycads vessel possess larger perforations.

The diameter of annular and spiral vessels is 10.03 ~ 12.69 μm, The diameter of scalariform vessel is 19.22 ~ 25.32 μm, The diameter of pitted vessel is 19.81 ~ 32.06 μm. The cell wall of parenchyma cells only consist of primary wall are not dissolved by enzymes[14], therefore they preserve their integrity of thin membrane wall, they are without perforations in their cell wall (Figure 1A), perforations can be seen only in trachea.

*Bowenia serrulata* In the pinna material, scalariform vessel, pitted vessel and reticular vessel were seen (Figure 2A – D), many perforations in end wall and lateral wall. Some lateral wall perforations were so large, that ornamentation and perforations in the interior walls were seen very clear (Figure 2B, D). These vessels possess longer and wide perforation, the transverse septas or bars consist of secondary wall are more longer, reach 14 ~ 17 μm. In some vessels, many perforations locate in one or two sides of lateral wall, but other section of walls have not perforation (Figure 2C), some vessels have already formed perforation in several sides of lateral wall (Figure 2D). The phenomenon was seen that scalariform perforation in a side, pitted perforation in another side of lateral wall (Figure 2B).

The diameter of scalariform vessel is 18.30 ~ 23.28 μm, the diameter of pitted vessel is 17.64 ~ 28.16 μm. the diameter of reticular vessel is 25.41 ~

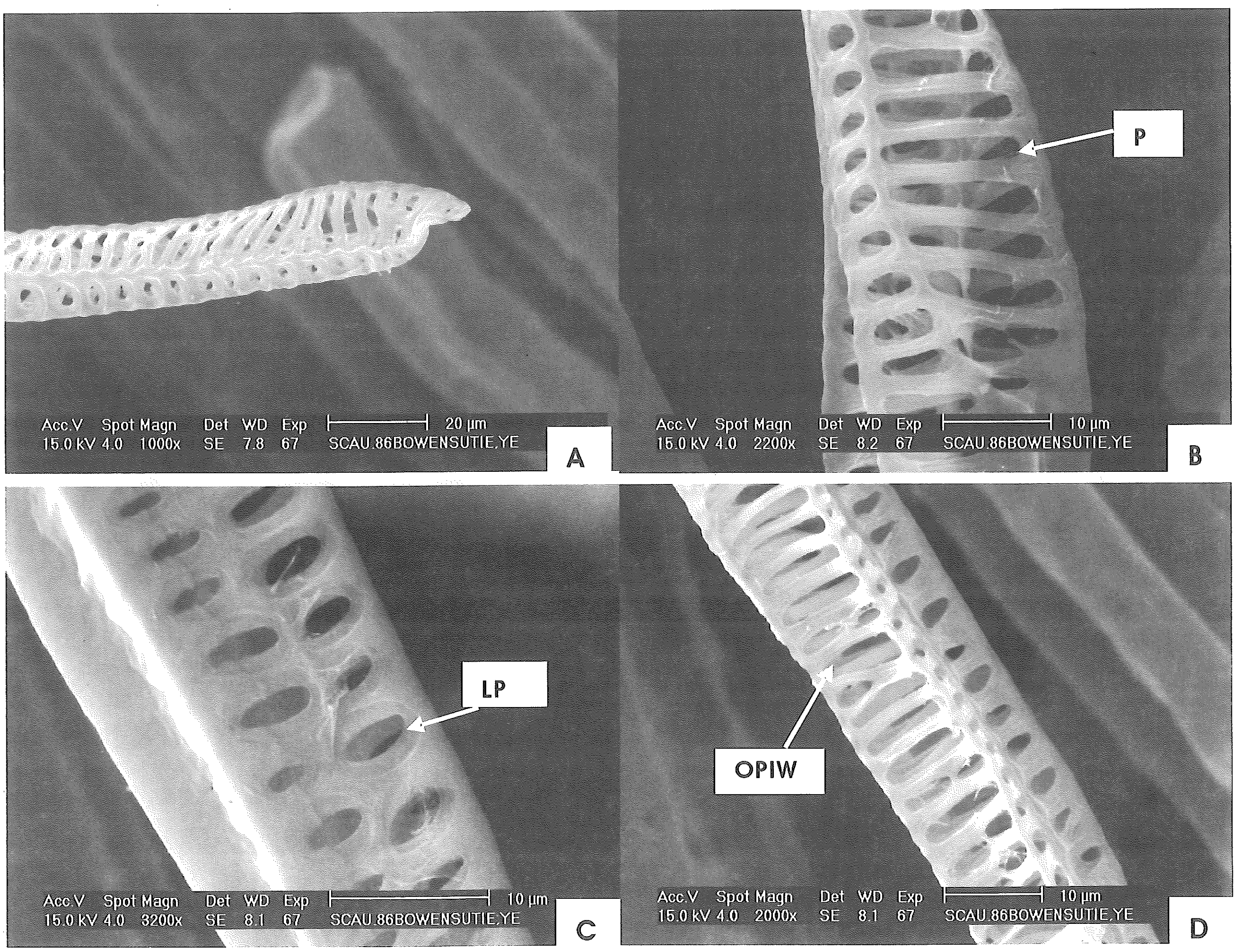


Fig. 2　SEM photographs of vessel elements of pinna of *Bowenia serrulata*

A: A reticular vessel element which have a horizontal end wall with a little tail, there more perforations locate in lateral wall. B: Showing scalariform vessel element, some long perforations locate in end wall. (P: Perforation). C: A pitted vessel element which possess some pitted perforations in lateral wall. (LP: Lateral wall perfration). D: A scalariform vessel element, there is some scalariform perforations in lateral wall (OPIW: Ornamentations and perforations in the interior walls)

39.22 μm. Some end wall are inclined perforation plates (Figure 3A & F), the degree of inclination is small in many vessels (Figure 3F). Some end wall of vessels are near horizontal or horizontal (Figure 2A; Figure 3C). Many of vessels are rectangle or polygonal. In end wall and lateral wall, some primary wall

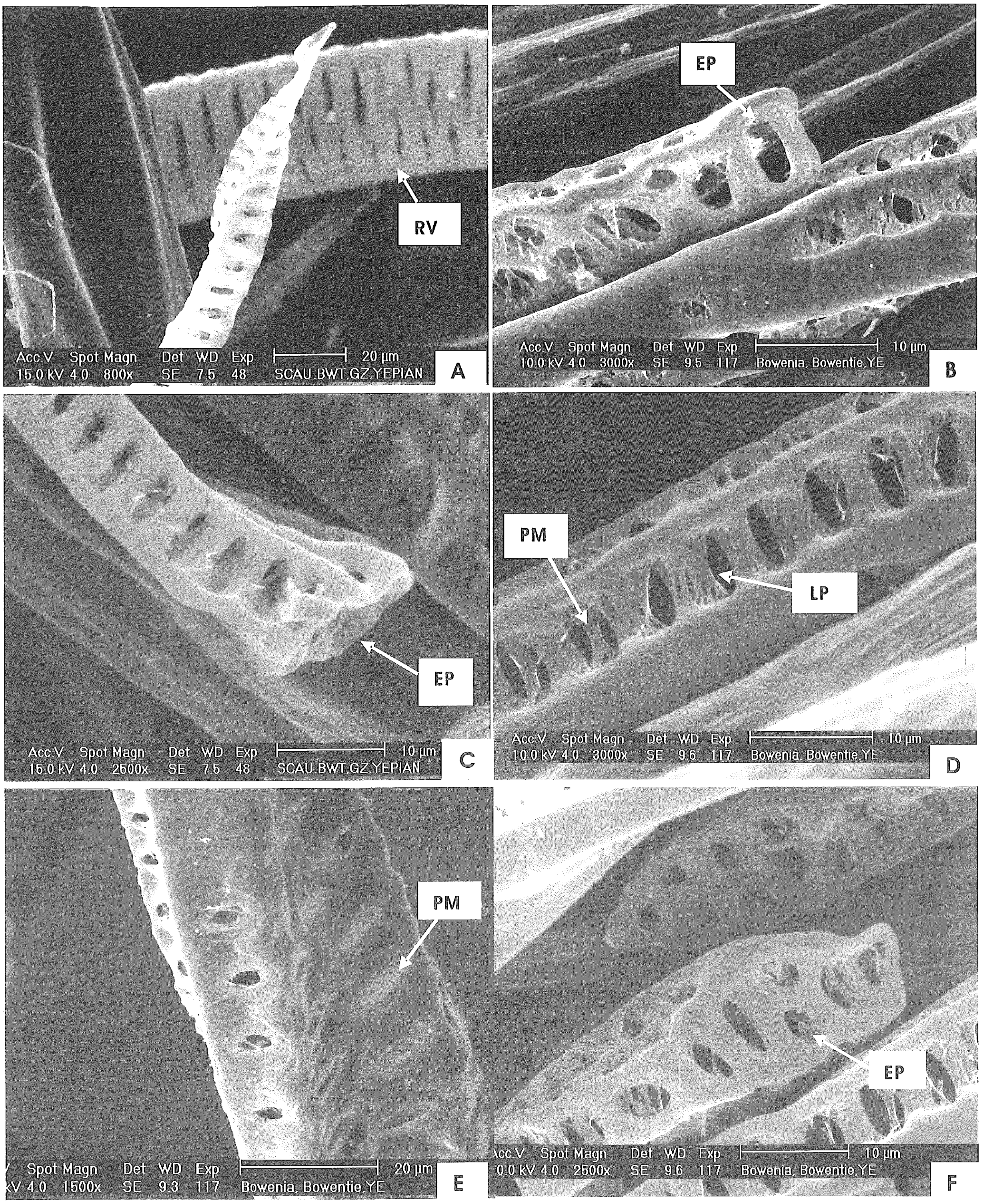


Fig. 3 SEM photographs of vessel elements of pinna of *Bowenia serrulata*

A: A scalariform and a reticular vessel element, showing the inclined perforation plate. ( RV: reticular vessel element ). B: Two pitted vessel elements, to show the inclination perforation plate of end wall, there are several perforations in the plate, and some threadlike remnants of primary wall in the periphery of perforations. C: Several scalariform – pitted vessel elements, to show their horizontal end wall. (EP: End wall perforation plate ). D: A pitted vessel element, it has some pitted perforations in lateral wall, there is some threadlike or mesh – like remnants of primary wall in the periphery of perforations. ( PM: Primary wall membrane; LP: Lateral wall perforation ). E: A pitted vessel element, some sites have formed perforations and some pits preserve still entire primary wall as a layer membrane in periphery of ornamentation which consist of secondary wall. F: Several pitted vessel elements, to show their inclined end wall perforation plates, there are six to seven perforations in the plate which wrap in outside of vessel have not been dissolved completely by enzymes, therefore preserve their some threadlike or mesh-like remnants of primary wall in the periphery of perforations (Figure 3B, D, F) [13, 15]. But there have formed some small perforations which only over a half in entire size, then these remnants complete disappearing, they will form large and entire perforations. The length in many perforations which located end wall or lateral wall of scalariform vessel and reticular vessel have already reach 15 μm some approach 20 μm , some reticular vessel are over 20 μm (Fig. 3 A), and width are 5 ~ 7 μm.

In some vessel elements, a portion of vessel wall have formed perforation, or remain few membrane remnants in the outside of perforation, and other sites preserve still entire primary wall as a layer membrane in periphery of ornamentation which consist of secondary wall (Figure 3E).

*Dioon edule* In pinna, annular, spiral and pitted vessels were seen. In some spiral vessels, more perforations have formed in lateral wall, some sites still preserve the membrane remnants in the periphery of perforations, but some spiral or annular vessels preserve still a more entire primary wall in the periphery of vessel elements (Figure 4A). Their end walls are mainly inclination shape (Figure 4B & D). In pitted vessel, they possess a more inclined end wall and some perforations in several sides of wall. Some surfaces of perforation preserve some threadlike remnants (Figure 4B).

The diameter of annular and spiral vessels is 6. 70 ~ . 62 μm, the diameter of pitted vessels is 10. 16 ~ 21. 29 μm.

Some end wall of spiral vessels possess a inclination perforation plate, and there are several large perforations in the plate (Figure 4D). The cell wall of parenchyma cell such as palisade tissue of assimilating tissue which only consist of primary wall are not dissolved by enzymes, without any perforations in the cell wall, therefore preserve their integrity of membrane-like wall (Figure 4C).

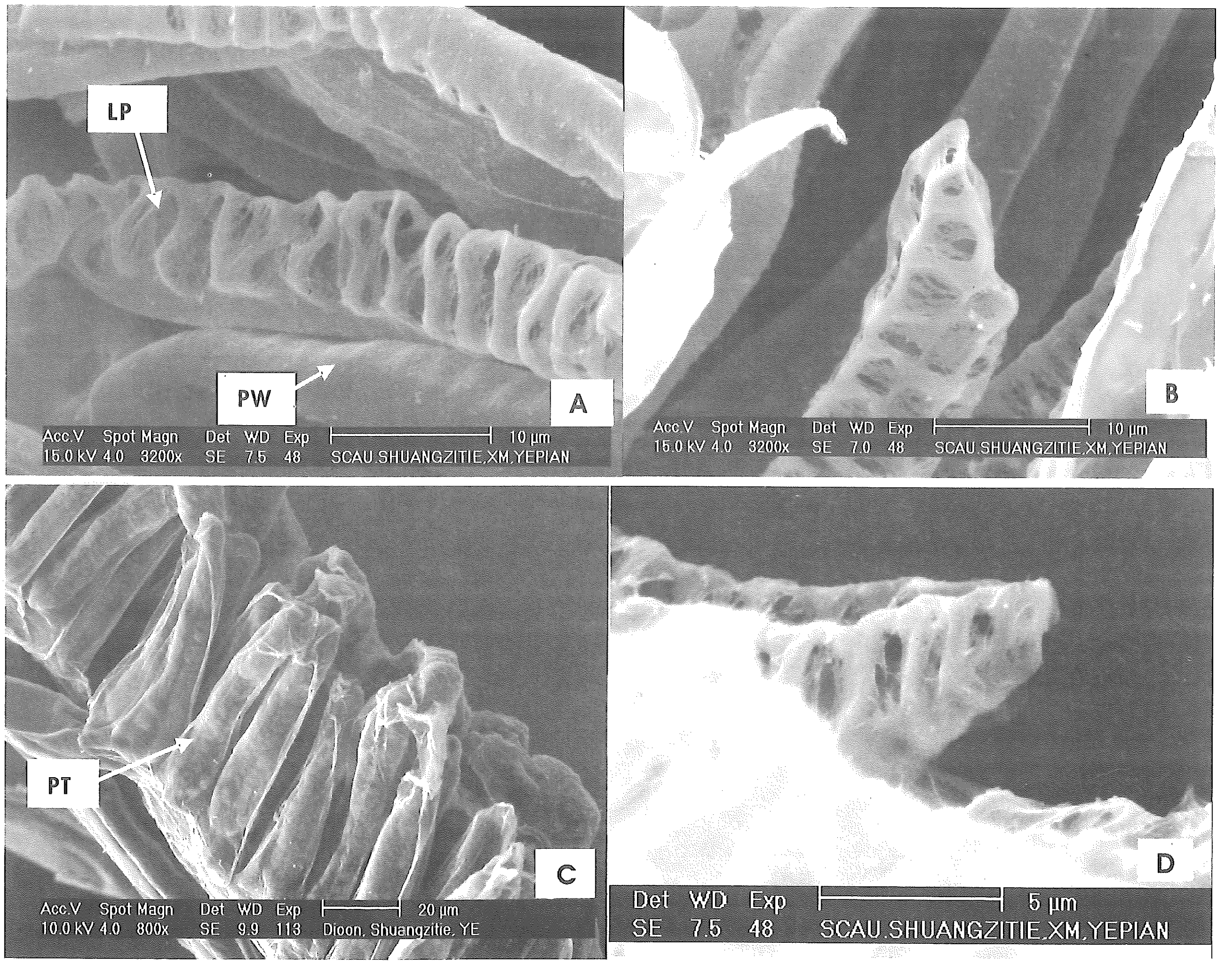


Fig. 4　SEM photographs of vessel elements of pinna of *Doon edule*

A: Several spiral vessel elements, their lateral wall have form some perforations with some threadlike membrane remnants of primary wall. LP: Lateral wall perforation, PW: primary wall ). B: A pitted vessel , there are some perforation with some threadlike or mesh – like membrane remnants consist of primary wall in the end wall. C: The parenchyma cell of palisade tissue of assimilating tissue, they preserve the integrity of membrane wall consist of primary wall. ( PT: palisade tissue ). D: A scalariform vessel element which possess some perforations in end wall

**Macrozamia longispina** In the root, scalariform, pitted, reticulate and reticulate-pitted vessels were observed, and they are more near circular and few near polygonal. Some are near the shape of flat. In pitted vessel, there are several perforations in the end wall perforation plate (Figure 5A), and the perforation plate is inclination shape. A part of pitted vessel are near flat shape, the perforations in the two sides, even if the end wall is also the situation, while it show a twisting shape (Figure 5 B). The phenomenon is widely extant that more perforations have formed in the lateral wall of a vessel element, and there is still very few perforations in another element of pitted vessel, the pit membrane is still preserve entire membrane in the outside of vessel (Figure 5B & C).

Some end walls possess some reticular perforations, and the inclined perforation plate have a tail (Figure 5C). Several adjacent elements of pitted and scalariform vessel showed the phenomenon that a vessel element preserve still threadlike membrane remnant of primary wall in the periphery of perforation, another a vessel element have formed entire perforations (Figure 5D). The result indicates that the vessel element differentiation is not asynchronous even if in same tissue.

The diameter of scalariform vessel is 14.72 ~ 19.41 μm, diameter of pitted vessel is 12.87 ~ 24.22 μm, the width of pitted vessel of flat shape is 23.67 ~ 25.96 μm, the diameter of reticular vessel is 15.64 ~ 22.79 μm.

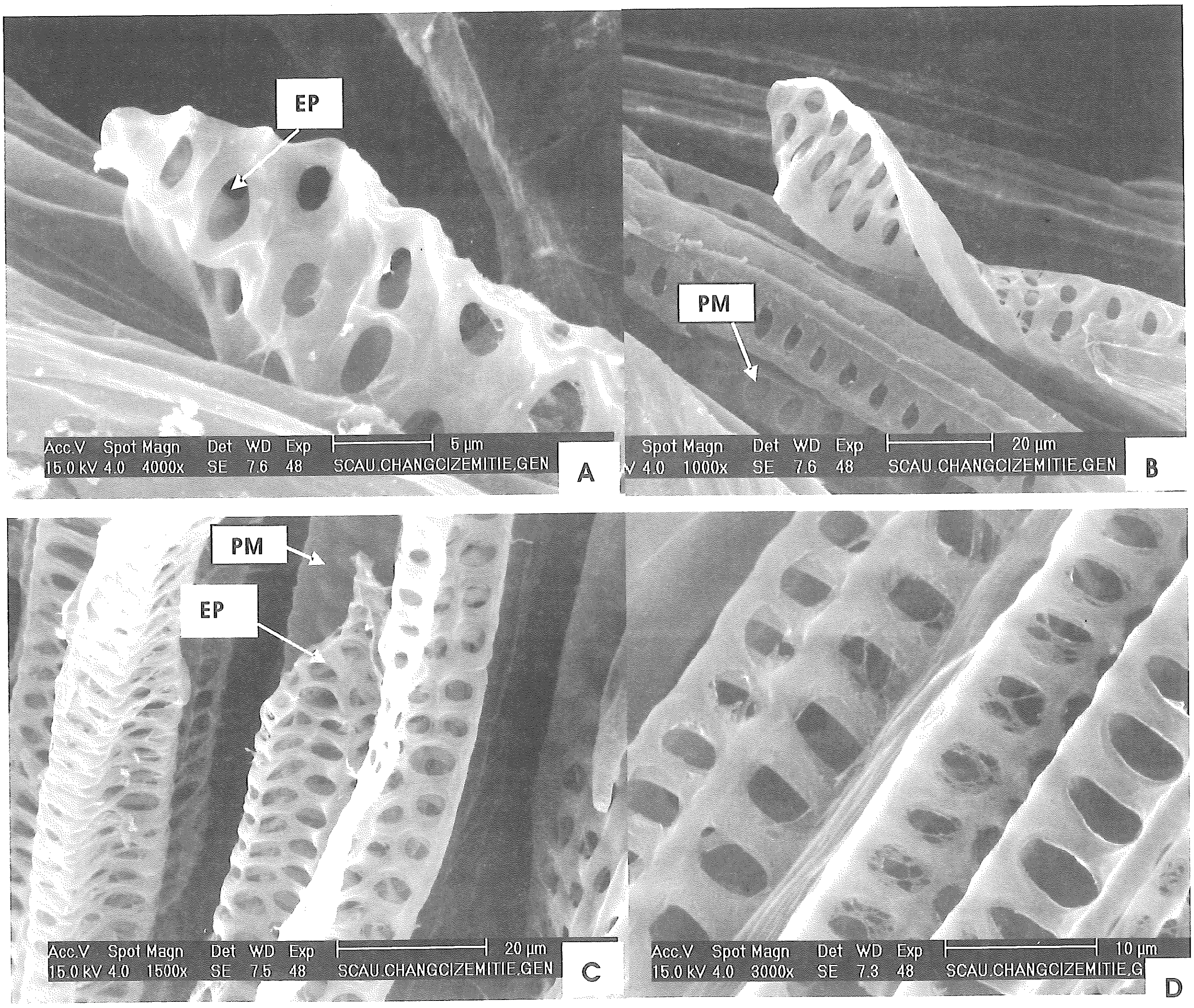


Fig. 5 SEM photographs of vessel elements of root *Macrozamia longispina*

A: A pitted vessel element, showing the inclined end wall perforation plate with several perforations in the plate. (EP: End wall perforation plate). B: Several pitted vessel elements, showing a flat shape vessel element with some twist. Another a vessel element preserve still entire membrane of primary wall in the outside of vessel. (PM: Primary wall membrane). C: Some pitted and reticular vessel elements, to show a perforation plate of end wall, with a little tail, their lateral wall have more perforations and some vessel elements preserve still entire membrane of primary wall in the outside of vessel. D: Several pitted and scalariform vessel elements, there are some entire perforations and some threadlike or weblike membranes still remain in the periphery of perforations

Michelia alba　In the leaf blade, the spiral, scalariform and pitted vessel were seen, the species have the distinct specialty that many vessel elements are flat shape or near flat - band shape. In some scalariform vessel elements, the end portion are flat like a slice and acute shape, there is without end wall, only possess a margin, their perforations adjoin each other only in lateral wall in two sides or several sides, these end wall have not inclined plate in top (Figure 6A & B). This character is more primitive.

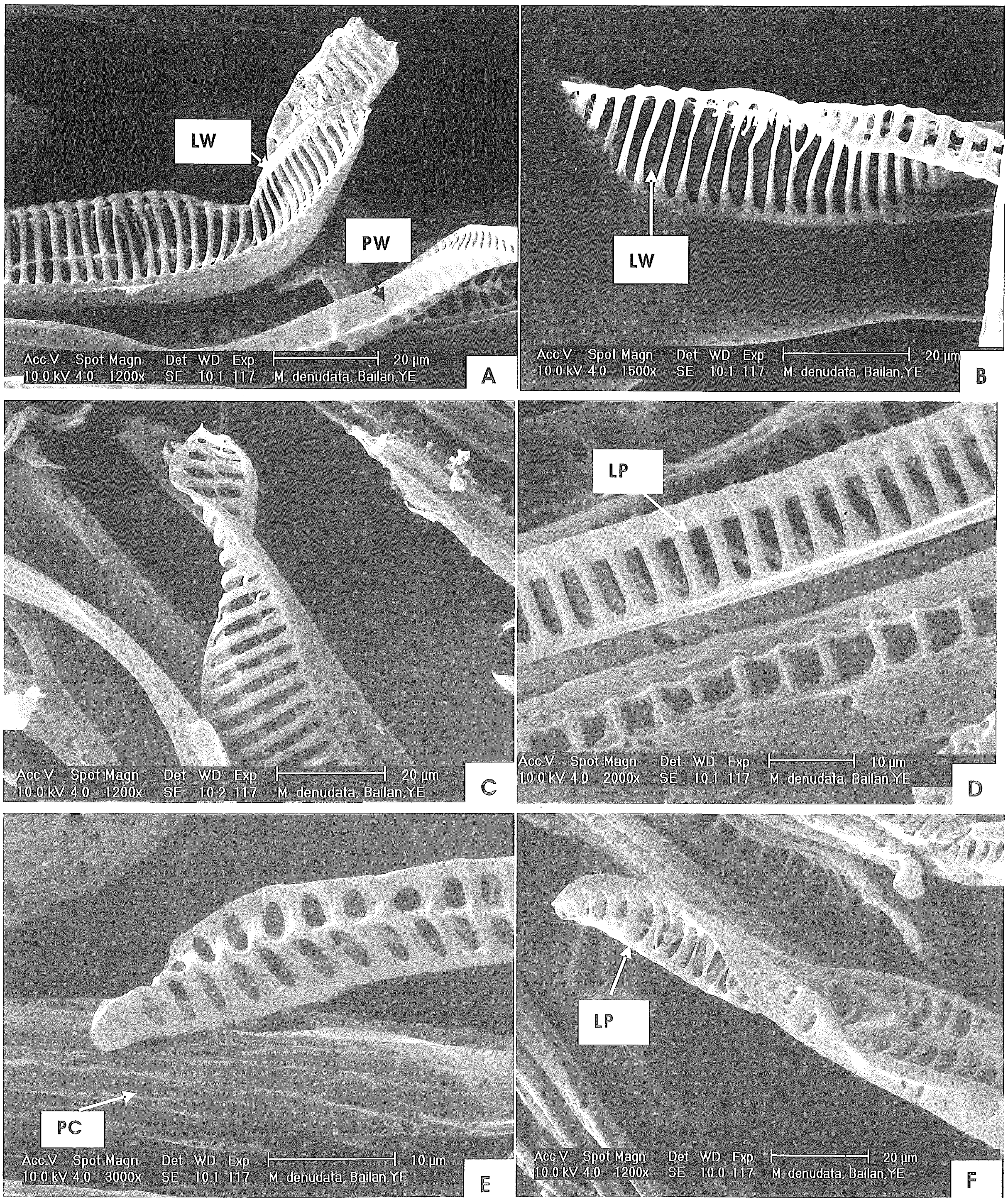


Fig. 6　SEM photographs of vessel elements of leaf blade of *Michelia alba*

A: Several scalariform vessel elements, showing their flat and band vessel elements with a bend shape, these end of elements have not end wall, only have perforations in lateral wall; (LW: Lateral wall, PW: primary wall). B: A scalariform vessel element, to show the near end portion there is a twist shape with some perforations in lateral wall, end is acuate, without end wall, only have a marge. C: A scalariform vessel element, showing the flat and twist shape and without top perforation plate in end, only having some perforations in the two sides of lateral wall. D: A annalar and a scalariform vessel elements, their lateral wall have some perforations. (LP: Lateral wall perforation). E: A pitted vessel element and some parenchyma cells only consist of primary wall which preserve their integrity of wall of thin membrane shape. The vessel element possess a acuate end portion, the perforations locate in several sides of lateral wall. (PC: parenchyma cell). F: A pitted vessel element, to show the flat and band shape, there are more perfrations in the two sides of lateral wall

These scalarifrom perforations distribute in end portion of lateral wall and other sites. Some vessel elements formed perforation only in a side or two sides, but other side have not perforation in lateral wall, still remain primary wall on the surface of lateral wall (Figure 6A). Some scalariform vessel elements like a flat band shape, their end portion is acute, with a twisting shape, their end portion have not also inclined plate in top, only have several lateral wall sides there are many perforations (Figure 6C, F). In the site there is longer distence to end portion, are formed many perforations in the lateral wall. Some ornamentation and large perforations can be seen on the interior wall of the vessels (Figure 6D & E). The cell wall of parenchyma cells only consist of primary wall are not dissolved by enzymes, wherefore preserve their integrity of wall of thin membrane shape (Figure 6E).

The diameter of annular and spiral vessels is 7.62 ~ 8.79 μm, the width of scalariform and pitted vessels of flat - band shape is 24.81 ~ 26.73 μm, the thickness is 8.74 ~ 12.62 μm; the diameter of scalariform and pitted vessels of near polygonal is 12.21 ~ 16.46 μm.

*Amygdalus persica* In the leaf blade, many annular, spiral and reticular vessels were observed. The spiral vessel have formed some perforations in lateral wall (Figure 7A), and some end wall of reticular vessel element is more inclined shape. The degree of inclination is greater, with simple perforation plate in its end wall and with a little tail, in some vessel elements of reticular vessel have formed some reticular perforations in lateral wall (Figure 7B).

The diameter of annular or spiral vessel is 8.72 ~ 12.67 μm, the diameter of scalariform vessel is 14.37 ~ 20.64 μm, the diameter of reticular vessel is 21.73 ~ 28.62 μm.

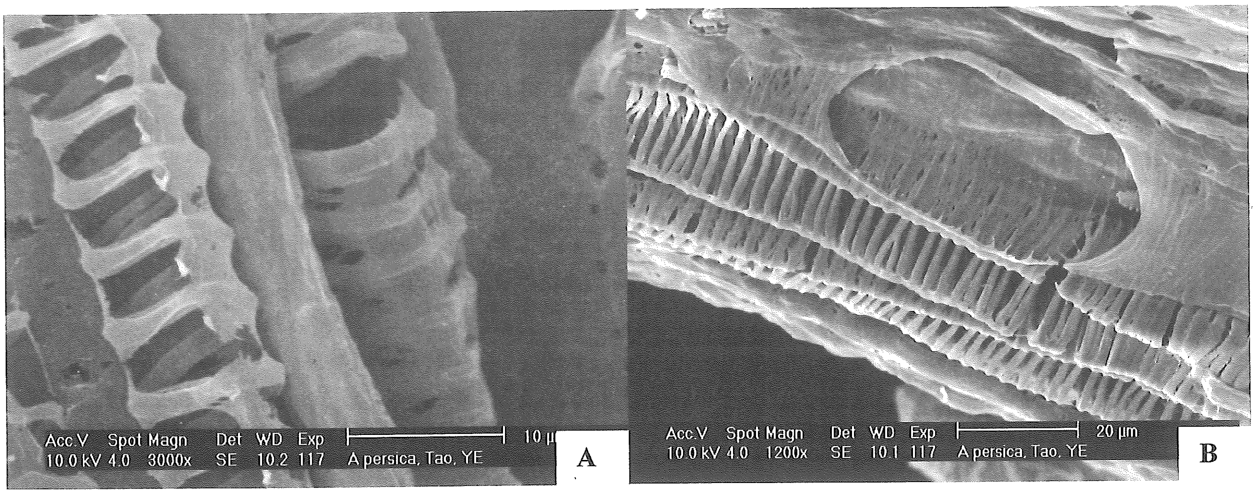


Fig. 7 SEM photographs of vessel elements of Leaf blade of *Amygdalus persica*

A: Several spiral vessel elements, their lateral wall have some perforations. B: Sveral reticular vessel elements, to show the the inclined end wall with a simple perforation plate and some perforations in their lateral wall

## 3 Discussion

Same as angiosperm, the structural types of the vessels of species in four genera of two families of cycad have a plentiful variety, and consisting of annular, spiral, scalariform, reticulate, pitted vessels and scalariform-pitted and reticulate-pitted vessels of transitional types.

The perforation plate of every end wall of cycads is very well developed, having large perforations. Some multiple perforation plates have several quite large perforations, but only a few have more than ten perforations. Compared with the characteristics of many species of the more primitive angiosperms whose vessel end walls have dozens, more than 100 and even 200 small perforations [13, 15-17], thus structures of the cycads vessels promote the rapid transport of large amounts water, as well as inorganic and essential organic nutrients [18].

The theory of comparative morphology suggests that more primitive vessels are inclination and possess a clearly and long pointed tail. Conversely, the vessel elements are more evolved and their end walls have gradually become horizontal or near horizontal and basically have not a tail [17, 19]. Compare with *Michelia alba* and *Amygdalus persica*, cycads vessel general possess a lesser inclination degree of end wall perforation plate, their end wall are more near horizontal than *Michelia alba* and *Amygdalus persica*, indicate that these vessels of cycads genera are the situation of more advanced in this index.

In the past, since observation is limited in light microscope, only focus to look whether have large single perforation which then many bars between some small perforations disappear and combine to a large perforation in perforation plate of end wall, therefore ever widely thought that fern is most primitive vascular plant, and they have not vessels. But with research increase and used electron microscope, vessel has been discovered in fern, and gradually increases families and genera. Now vessels have been discovered in some ferns which be thought most primitive taxa, such as Selaginella genera of Selaginellaceae, *Equisetum* genera of Equisetaceae and more evolutionary taxon such as Pteridium genera of Pteridiaceae, *Phyllitis* genera and *Marsilea* genera of Marsilaeaceae etc. [20-23]. Therefore the high and woody cycads possess vessel is a necessity which plant adapt to environment and natural evolutional phenomenon.

Many vessels of *Michelia alba* have not top plate in their end portion, only have acute or a line shape margin in end, means that they without end wall; end portion only consist of wider two lateral wall of flat and like band shape, adjoin elements of vessel connect each other rely on only the perforations which locate in these lateral walls, their end is more acute or point. These characters are more primitive character of vessel, these features are have no seen report in past. Past viewpoint basically thought that vessel elements certainly preserve a top plate is very inclination, more inclination, near horizontal or horizontal. In fact, the phenomenon is existence which vessel element without formed any end wall or call top plate as even if it is very inclination, adjoinning vessel elements connect each other rely on the perforations of several lateral walls, and thus vessel is still having a large ability in conduction, even exceed the ability which possess only a top plate in end wall because of several side surface of lateral wall and their more or large perforations, furthermore thus structure can serve a important action that an vessel element can connect two to several other vessel elements in longitudinal direction. It is the reason that even if these features are more primitive in structure, they can preserve still in angiosperm or cycads.

The research show the some vessel element of *Michelia alba* of angiosperm and cycad possess twisting shape, this structure can make adjoin several vessel elements connect in a little shape of twist together, thus structure probably can make it serve well conduction and stronger capability of mechanical support in same time.

In this study, perforations can be seen only in trachea, the cell wall of parenchyma cells only consist of primary wall have not been seen any perforation, indicated that enzyme action are specificity which only act to intercellular layer (middle lamella) of the tissue of tracheary elements or parenchyma cells, and not make dissolved action to membrane-like primary wall , wherefore parenchyma cells preserve integrity of thin membrane wall.

The theory of comparative morphology suggests that smaller diameters of vessel, are more ancestral [24-27]. Present research shows that the diameter of vessel elements of cycads and two genera of angiosperm is same in size, account for the extent of evolution is near or same in the index.

As same as many angiosperms [12,13, 15], the phenomenon that some vessel elements of cycads preserve still threadlike or mesh-like membrane remnant of primary wall in the periphery of perforation, and can seen the process that primary wall gradually formed small or over a half perforation, and then these remnants complete disappearing formed large and entire perforation.

Because primary wall of every tracheary element wrap in most periphery of the cell, afterward the secondary wall of lignification and corkification form in inside primary wall, and they form various ornamentations such as the ornamentations of annular, scalariform, pitted, reticulate and some transitional types of scalariform-reticulate etc. locate in interior layer of primary wall; if the tracheary element is pitted tracheid or vessel element, the pit cavity of simple or bordered pits which were formed by secondary wall are located in inside primary wall, the surface of tracheid or vessel element have not pit or pore as entrance, because these pits periphery wrap a layer membrane of primary wall [2,19, 28, 29]. in the place of between two adjoined tracheary elements is intercellular layer (middle lamella) consist of pectin, two tracheary elements divorce from this layer then experimental treat [10-11]. Therefore if there is perforation in tracheary element, its structure which were seen in scanning electron microscope (SEM) is dark and deep hole, if those sites without perforation, the primary wall still wrap in the cell surface, these walls' structure which were seen in SEM are bright and preserved level and smooth surface.

The research shows that like many angiosperms, in *Michelia alba* and *Amygdalus persica* and four genera of cycads, the lateral wall of the vessel elements possess perforations, and these perforations link up between vessel elements in the side surface, so that these elements not only communicate longitudinally and have powerful longitudinal transport ability, but also have a strong capability for transporting water, as well as inorganic and organic nutrients in a transverse plane [2,19]. For example, the lateral wall perforations of tomato

(*Lycopersicon esculentum*) [30]. and so on.

The multiple perforation plates such as the scaraliform perforation plate and the reticulate perforation plate are primitive, and from them evolved the simple perforation plate that possesses only one perforation in the end wall [17, 19, 31].. This research results showed that some species of cycads have evolved to form simple perforation plates in end walls, indicating that the vessels of cycad are quite evolutionally advanced.

Vessels evolved from tracheids, and they have formed many perforations in the end wall and lateral wall, so the vessels are able to transport about ten times more water and nutrients than tracheids in a given length of time [31] The plentiful types of vessels discovered in these genera of cycads, and compare with the vessel of two families of angiosperm, thereby cognized the levels of evolution of cycads vessel element, it is important to understand the mechanism which these most primitive seed plants extant how to adapted to harsh environments and reveal the secret how cycads survive and procreate undergo after a very long geological period.

## References:

[1] DEHGAN B, YUEN C K H. Seed morphology in relation to dispersal, evolution and propagation of *Cycas*[J]. Botanical Gazette, 1983,144:412 – 418.

[2] FAHN A. Plant anatomy[M]. 4th ed. Oxford, UK: Pergamon Press, 1990.

[3] PEARSON L C. The diversity and evolution of plants [M]. Boca Raton and New York, London, UK and Tokyo: CRC Press, 1995.

[4] STERN K R. Introductory plant biology[M]. Dubuque, Iowa, USA: Wm. C. Brown Company Publishers, 1982.

[5] WU Xianjun, LI Ping, ZHAO Zenju, et al. Studies on the biological characteristics of *Cycas panzhihuaensis*. I. Morphology and anatomy of vegetative organs[M]. Chi Bull Bot, 1995(Spesial column):38 – 40.

[6] YANG Guanxiu, CHEN Fen, HUANG Qisheng. Palaeobotany[M]. Beijing: Geological Press, 1994.

[7] HUANG Yuyuan, ZHANG Hongda. The brief report on discovery of vessel in cycads[J]. J Guangxi Agric and Biol Science, 1999, 18: 161 – 162.

[8] LIN Jianzhao, HUANG Yuyuan. Discovered vessel in cycads again[J]. J Guangxi Agric and Biol Science, 1999, 18(3): 332 – 335.

[9] HUANG Rong, WU Jun. Vessels discovered in *Cycas panzhihuaensis* L. Zhou et S. Y. Yang[J]. Journal of Sichuan University: Natural Science Edition, 2007, 44 (2):430 – 433.

[10] LI Zhengli. Technique of plant microscopic mount[M]. 2nd ed. Beijing: Science Press, 1987.

[11] ZHENG Guochang, GU Zuping. Biomicrological technique[M]. 2nd ed. Beijing: Higher Education Press, 1993.

[12] NAKASHIMA J, TAKABE K, FUJITA M, et al. Autolysis during in Vitro tracheary element differentiation: formation and location of the perforation[J]. Plant & Cell Physiology, 2000, 15: 1267 – 1271.

[13] CARLQUIST S, SCHNEIDER E K. Perforation plate pit membrane remnants and other vessel details of Clethraceae: primitive features in wood of ericales[J]. Int J Plant Sci, 2004. 165, 369 – 375.

[14] ASPEBORG H, SCHRADER J, COUTINHO PM, et al. Carbohydrate – active enzymes involved in the secondary cell wall biogenesis in hybrid aspen1[J]. Plant Physiology, 2005, 137(3):983 – 997.

[15] SCHNEIDER LE, CARLQUIST S. Perforation plate pit membrane remnants in vessels of Sarraceniaceae: possible indicators of relationship and ecology[J]. Journal of the Torrey Botanical Society, 2004, 131(1):1 – 7.

[16] SCHULTE P J. Water flow through a 20 – pore perforation plate in vessels of *Liquidambar styraciflua*[J]. Journal of Experimental Botany, 1999, 50(336): 1179 – 1187.

[17] GU Angen, LU Jingmei, WANG Lijun. Evolutional morphology of vascular plants[M]. Changchun: Jilin Science Technology Press, 1993.

[18] ODA A, SAKUTA C, MASUDA S, et al. Possible involvement of leaf gibberellins in the clock – controlled expression of XSP30, a gene encoding a xylem sap lectin, in cucumber roots1[J]. Plant Physiol, 2003, 133: 1779 – 1790.

[19] ESAU K. Anatomy of seed plants[M]. 2nd ed. New York: John Wiley & Sons, 1977.

[20] BIERHOST D W. Vessel in *Equisetum*[J]. Amer Jour Bot, 1958, 45: 534 – 537.

[21] BLISS M C. The tracheal elements in the ferns[J]. Amer Jour Bot, 1939, 26:620 – 624.

[22] WHITE R A. Vessel in root of Marsilea[J]. Science, 1961, 133: 1073 – 1074.

[23] GU Angen, LIU Yixian On the discovery of vessel in phyllisin[J]. Acta Botanica Sinica, 1987, 29(4):377 – 378.

[24] YU Hongcheng. The relation with the evolution of second xylem and systematic development of plants [J]. Acta Bot Sin, 1954, 3: 183 – 195.

[25] YU Hongcheng. The evolutionary tendence of second xylem of gymnosperm [J]. Acta Phytotaxia Sin, 1981, 19:179 – 185.

[26] ZHOU Yin, JIANG Xiaomei. Wood anatomy and ultrastructure of gymnosperm of China[M]. Beijing: Forestry Press of China, 1994.

[27] WANG Faxiang, LIANG Huibo, CHEN Tanqing, et al. Cycads in China[M]. Guangzhou: Guangdong Science and Technology Press, 1996.

[28] CRONQUIST A C. Basic botany[M]. 2nd ed. New York: Harper & Row Publishers, 1982.
[29] LI Yanghan. Botany: First Vol[M]. 2nd ed. Beijing: Higher Education Press, 1986.
[30] NIEVES F G, CARVAJAL M, OLMOS E. Graft union formation in tomato plants: Peroxidase and catalase involvement[J]. Ann Bot, 2004, 93:53 - 60.
[31] SPERRY J S. Evolution of water transport and xylem structure[J]. Int J Plant, 2003, 64:115 - 127.

# 苏铁类植物 4 个属的导管与被子植物导管结构特征的比较研究

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**摘　要：** 对苏铁属的台湾苏铁（*Cycas taiwaniana*）、波温铁属的细齿波温铁（*Bowenia serrulata*）、双子铁属的双子铁（*Dioon edule*）叶的羽片，大泽米铁属长刺大泽米铁（*Macrozamia longispina*）的根，以及被子植物的白兰（*Michelia alba*）和桃（*Amygdalus persica*）的叶组织进行了电子扫描显微镜的观察研究，结果表明，2 个科 4 个属苏铁植物的导管类型是丰富的，导管的结构组成方式、发育过程与被子植物是一致的；例如导管端壁倾斜度等结构特征显示，一些被子植物导管的结构比苏铁类植物的要原始。在被子植物的导管中有许多为端部呈扁平的带状，而且还带有扭曲状，没有端壁，只有两面的侧壁及另两侧的很窄的边，在带状的侧壁上具有许多的穿孔。这在过去未见有报道。对苏铁类植物导管的认识，对于我们进一步了解现存最原始的种子植物对严酷环境的适应机制和进化状况，对于采取有效措施保护这些珍稀的植物类群，以及对于结构植物学、植物进化和生态学等研究方面均具有重要的意义。

**关键词：** 苏铁类植物；导管；被子植物；演化；结构特征；适应性
**中图分类号：** Q941；Q944.6

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# Construction of Chimerical Fungal Phytase and its Resistance to Proteolysis

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**Abstract**: Chimera Ⅰ, Ⅱ and Ⅲ were created by stepwise substitutions of DNA fragments encoding region 1 - 47, 178 - 237 and 338 - 381 from Aspergillus niger NRRL 3135 phytase (Anp) gene for their counterparts in Aspergillus fumigatus phytase (Afp) gene, expressed in Pichia pastoris, and then purified. All chimera and parent phytases resisted to pepsin. Afp and all chimeras lost 13% ~ 23% phytase activities at $m$(trypsin) /$m$(phytase) ratio of 0.1, while Anp was thoroughly inactivated. However, SDS - PAGE of trypsin digestion results suggested serious proteolysis of Chimera Ⅱ and Ⅲ. But nondenaturing PAGE analysis showed they still kept structural intact in nature state after trypsin treatment. Chimera phtases also possessed novel pH profiles and thermo stability different from Anp and Afp. These results demonstrated the feasibility of improving fungal phytase via gene fragment repalcement.

**Key words**: phytase; *Aspergillus fumigatus*; *Aspergillus niger*; proteolysis; protein engineering