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IDENTIFICATION OF MALE AND FEMALE *Cycas revoluta* Thunb. BEFORE MATURITY USING MORPHOLOGICAL AND ANATOMICAL FEATURES

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Abstract

This paper reports sex determination of cycad (Cycas revoluta Thunb.) before maturity and cone formation. Sex determination in cycad is possible only after maturity and cone formation. In ideal conditions, it takes at least 10 to 12 years for cycad to reach maturity. In this research, two-years-old male and female off-shoots of cycad were separated from the adult male and female plants and were treated in natural conditions, uniformly. Morphological characteristics of male and female plants were visually compared. Anatomical features were studied on the sections prepared from primary leaf rachis, leaflet and root using light microscope. According to the Discriminate Analysis (DA) method, differences between morphological characteristics of male and female plants such as leaf rachis diameter, leaflets width, leaflets distance on the rachis, leaflets angle on the rachis, and roots diameter and length, were significant. The arrangement of leaves in one third of middle part was bipinnate with opposite leaflets and bipinnate with alternate leaflets in male and female plants, respectively (new leaflets initiate forms before and after complete formation of previous leaflets in female and male plants, respectively). Anatomical studies showed that the difference between the lengths of lower stomata in male and female leaflets were significant. Stoma type in cycad leaf is actinocytic.

Key words**:** *Cycad, Ornamental plants, Sex determination*

INTRODUCTION

Cycas is the only genus of the Cycadaceae family and includes about a hundred species, with the most prominent specie *Cycas revoluta*. *Cycas revoluta* is the second species in the genus *Cycas* and it is the only species present in Asia (Liu et al., 1991; Donaldson, 2003; Hill, 2004). This plant has a long history and is popular because of beautiful pinnate and evergreen leaves. *Cycas revoluta* is used as an ornamental pot plant, and in landscape and environmental design. The habitat of *Cycas revoluta* is southern Japan at 31′ north latitude including Ryukyu, Mitsuhama and Satsuma islands in the Southern island of Kyosho (Stevenson, 1990). *Cycas revoluta* is one of the most widely grown cycads in subtropical regions or in greenhouses in colder areas (Donaldson, 2003). Cycads propagate by seeds, off-shoots, and stem cuttings and in tissue culture conditions (Jones, 1993; Rainald, 1998).

Cycad is a dioecious plant and its sex determination is possible only after maturity and cone formation. In ideal conditions, it takes at least 10 to 12 years for cycad to reach maturity (Raju and Jonathan, 2010). Thus, appearance and morphological identification of *Cycas revoluta* male and female plants is not possible until maturity. Therefore, sex determination takes place only after maturity and formation of flowers and cones. Assessment of male and female plants before maturity is necessary for breeding, reliable care and production planning. According to the literature, no study has been performed in terms of the identification of male and female trunks of *Cycas revoluta* before maturity. In various sources announced that the identification of male and female trunks of *Cycas* before maturity and cone formation is not possible (Raju and Henry, 2010). Thus, this study was performed to achieve valid and reliable diagnostic methods to identify *Cycas revoluta* male and female plants before sexual maturity.

To achieve this goal, anatomical and morphological features of *Cycas revoluta* in the vegetative growth period were evaluated.

MATERIALS AND METHODS

For morphological studies, off-shoot of matured *Cycas revoluta* which were characterized in terms of sexual and were grown under outdoor conditions. The samples were taken from Cycads breeding centers around the city of Rasht, Iran in early April, 2011. The two-year off-shoots in the physiological age were uniform and containing leaves but without roots. Separated shoots from the parental stock were disinfected with a fungicide. Leaves were cut from shoots to reduce surface evaporation and transpiration and then were planted in isolated pots with uniform soil composition containing a mixture of peat moss, sand and garden soil (1:1:1). To implement the plan, six pots from each male and female trunk were selected and randomly named. Then, the pots were randomly assigned into the 3 columns and 4 rows and were kept under suitable conditions. Plants were irrigated twice a week in summer and once a week in the fall and winter. Shoots produced roots during the spring and new leaves during August. Morphological studies were carried out in May 2012.

Measured morphological traits of male and female trunks of *Cycas revoluta* were: leaf rachis diameter, leaflet length, leaflets thickness, leaflet width, petioles diameter, distance between the leaflets on the leaf rachis, angle between leaflets and leaf rachis, shoots diameter, root diameter, root length, chlorophyll content, leaflet color, leaflets arrangement on leaf rachis, way to start up and formation of leaflets at the end of leaf rachis, the position of leaflet blades compared to the leaf rachis and the shape and color of the roots. To measure quantitative traits, digital caliper, ruler and chlorophyll content, manually chlorophyll meter (SPAD-502) was used. Qualitative study was performed as visual observation by the manual lens and light microscope.

The diameter of leaf rachis was calculated between leaflets 29 and 30 using a digital caliper. The length of the leaflets was calculated by a digital caliper on leaflets number 30. The thickness and width of leaflets were measured in the middle of leaflet number 30 using a digital caliper. The diameter of the petiole was obtained in the connection place of leaflets to rachis in leaflet number 30 using a digital caliper. Distance between leaflets in the interval of leaflets number 29 and 30 in leaf rachis was measured using a digital caliper. Measurement of the angle between leaflets (number 30) and leaf rachis was done using conveyor by hand after removing the other leaflets from leaf rachis. Diameter of the shoots in the place of largest diameter was obtained by a digital caliper. To measure the diameter of roots, secondary roots were used. Thus, diameter of secondary roots was measured at 3 cm from the terminal of the root. For determination of the amount of chlorophyll, leaflets were selected. For this purpose, the hand-held chlorophyll meter SPAD-502 was used. Shape, the position of blade leaflets in relation to the leaf rachis and leaflet arrangement was evaluated by direct observation (naked eye). To investigate initiation and formation of leaflet at the ends of leaf rachis, apical meristem of leaf rachis were observed continuously over several days. To assess the shape and color of the roots, they were washed with water and assessed by direct observations on male and female plants.

Anatomical studies on leaf rachis, leaflets and root was performed. The anatomical traits of leaf rachis of the male and female trunks of *Cycas revoluta* were the number of layers of the parenchyma cortex in rachis, stele diameter, the number of procambium layers, and number of vascular bundles, pericycle layer thickness, density and type of trichoms. Anatomical characteristics of leaflets rachis of male and female trunks were the number of upper and lower layers of parenchyma, type of stoma, upper and lower stomatal density, height of the upper and lower stoma, types of hairs and hairs density in upper and lower layers. Anatomical features of roots of male and female trunks were the number of cortex parenchyma layers, number of cambium layers, stele diameter, pericycle layer thickness and the number of vascular bundles.

For study anatomical characteristics of *Cycas revoluta*, at first the various organs were

sampled. Plant samples were fixed in a solution containing 10% formalin + 85% alcohol + 5% acetic acid. The transverse sections of leaf rachis, petiole and root were performed manually using a sharp blade. Samples were cleared by sodium hypochlorite and acetic acid. Samples were stained with Congo red and methyl blue. To study hairs in the leaf rachis and the stomata and hairs in the leaflets, living specimens without fixation was used. By removing the epidermis, the hairs on rachis and stomata hairs in the leaflets were examined. The prepared samples were photographed by Olympus camera DP71 attached to the light microscope. Identify the cells and different tissues on the taken images were performed. To evaluate the anatomical features of leaf rachis, one of the newly produced leaf rachis containing soft and young tissues was randomly selected and cut from the interval of leaflets number 15 and 16. To determine the presence or absence of the hairs in the rachis, epidermis was separated from the leaf rachis and the interval of leaf number 15 and 16 was selected and observed under a light microscope. Number of hair in one square millimeter from the rachis was counted. The leaflet number 30 was randomly assigned to evaluate its anatomy. To determine the type and density of stomata on leaflet, the upper and lower epidermis was separated from the selected leaflet number 30 and was studied by light microscopy. Type and density of stomata per square millimeter of a leaflet and length of stomata per micrometer was determined and photographs were taken from the stomata. Young roots were randomly selected to evaluate their anatomy.

RESULTS AND DISCUSSIONS

In this study, various morphological characteristics of male and female trunks of *Cycas revoluta* have been studied. The results have been showed in Tables 1 and 2. According to Tables 1 and 2, the difference between the diameter of leaf rachis in both male and female trunks of *Cycas revoluta* before the maturity is significant and indicated that the diameter of leaf rachis in male and female trunks is different. Contrary to our results, Stevenson (1993) reported that the leaf rachis in *Cycas* is convex-shaped and there is no any difference between the male and female trunks. Results showed that the difference in leaflets height between male and female trunks is not significant (Tables 1 and 2). Studies of Jones (1993) confirm our results. According to Tables 1 and 2, the difference between leaflet thickness in male and female trunks is not significant. Studies of Hill (2004) revealed that the thickness of leaflets at the trunks of the male and female in *Cycas* is the same and is variable from 0.3 mm in young leaves to 0.8 mm in older leaves.

Current investigation demonstrated that there is noticeable difference between leaflet widths at the mid-point of 30 leaflet trunks. Hill (2004) showed that leaflet width in male and female trunks of *Cycas revoluta* is equal and varies between 0.6 to 1.5 cm. Jones (1993) has reported that the diameter of node of leaflet varies from 2 to 4 mm (Jones, 1993).

Table 1 shows that there is no significant difference between petiole diameter in male and female trunks.

Distance between leaflets on the leaf rachis in the interval of leaflet numbers 29 and 30 on the trunks of *Cycas revoluta* were significant. Stevenson (1993) states that cycas leaf length in the trunks of male and female is alike and up to 200 cm. Also, the number of leaflets per leaf reaches 250.

The results of current study indicated that angle between leaflets and leaf rachis was up to 53 and up to 48 in female and male trunks, respectively. Study of Stevenson (1990) showed that male and female leaflets of *Cycas revoluta*, with an angle of about 45; are located on the rachis. The results obtained in our report are not consistent with those of Stevenson (1990).

According to Tables 1 and 2, the difference in shoot diameter is not significant.

Our findings is in accordance with study of Tang (1991). Study of these researchers on off-shoots removed from the lower parts of male and female stems showed that there is not any difference between the place of shoot formation, number and size.

Also, data obtained from the current study showed that the differences in the root diameter and size are significant. Hill (2012) reported that size, diameter and volume of root were

variable in cycads, ranging from very small branches to large branches and sub-branches. Our reports are consistent with the results of Hill (2012).

Difference in root length was significant. Root length was longer for female trunk than that of male trunk (Tables 1 and 2).

Table 1. Discriminate Analysis (DA) of means of groups for morphological characteristics of male and female trunks of *Cycas revoluta*.

Source of variances	Vilex ƛ	F	df_1	df_2	Sig.
Main axils diameter of leaf (X_1)	0.459	11.765	1	10	0.006^{**}
Length of leaflet (X_2)	0.832	2.017	1	10	0.186^{ns}
Leaflet diameter (X_3)	0.953	0.494	1	10	0.498^{ns}
Leaflet width (X_4)	0.111	80.112	1	10	0.000^{**}
Petiole diameter (X_5)	0.898	1.137	1	10	0.311^{ns}
Distance of leaflets (X_6)	0.147	58.209	1	10	0.000^{**}
Angle between leaflets (X_7)	0.314	21.797	1	10	0.001^{**}
Off-shoots diameter (X_8)	0.903	1.079	1	10	0.323^{ns}
Roots diameter (X_9)	0.177	46.593	1	10	0.000^{**}
Length of roots (X_{10})	0.240	31.743	1	10	0.000^{**}
Chlorophyll content in leaflets (X_{11})	0.995	0.054	1	10	0.822^{ns}

**: Significant at $\alpha = 1\%$, ^{ns}=Not significant

Table 2. Quantitative data of morphological characters in male and female trunks of *Cycas revoluta*

Morphological characters	Female	Male
Main axils diameter of leaf (mm)	3.21-3.51 ± 0.3 (3.81)	3.02-3.41 ± 0.39 (3.8)
Length of leaflet (mm)	73.98-85.05 ± 11.07 (79.515)	81.8-85.2 ± 3.4 (83.5)
Leaflet diameter (mm)	0.32-0.58 ± 0.26(0.45)	0.35-0.47 ± 0.12 (0.41)
Leaflet width (mm)	4.51-4.98 ± 0.47 (4.745)	3.77-4.04 ± 0.27 (3.905)
Petiole diameter (mm)	2.42-2.63 ± 0.21 (2.525)	2.25-2.63 ± 0.38 (2.44)
Distance of leaflets (mm)	5.33-6.41 ± 1.08 (5.87)	3.13-4.39 ± 1.26 (3.76)
Angle between leaflets (°)	47-53 ± 6 (50)	42-48 ± 6 (45)
Off-shoots diameter (cm)	5.05-6.15 ± 1.1 (5.6)	5.09-5.85 ± 0.76 (5.47)
Roots diameter (mm)	2.07-2.63 ± 0.56 (2.35)	3.08-3.92 ± 0.84 (3.5)
Length of roots (cm)	21.2-24.2 ± 0.3 (22.7)	15.5-20.4 ± 2.45 (17.95)
Chlorophyll content in leaflets	47.7-52.1 ±4.4 (49.9)	43.9-54.6 ± 10.7 (49.25)

Jones (1993) reported that the roots of cycads often are short and fleshy and do not tend to move into deep soil.

Young leaves in *Cycas* are soft and light green whilst older leaves are glossy, leathery and dark green (Stevenson, 1993). There is no significant difference in chlorophyll content of male and female trunk leaflets in *Cycas revoluta*.

Leaflet shape on male and female trunks was examined, observationally. Leaflets in both trunks are spear-shaped. Jones (1993) has reported that the leaflets in *Cycas revoluta* are numerous, sharp and spear. Leaflets have longitudinal veins and lack secondary and lateral veins. Cycas leaf is bi-pinnate with complete parts and edges (Ghahraman, 2003).

In our researches, leaflet arrangement on the rachis is divided into three parts. Leaflets are located in the both sides of rachis as alternate and opposite with no particular order.

In the middle third of the leaf, leaflet arrangement plays an important role in identifying male and female trunks of *Cycas revoluta* and is considered as one of the morphological differences between male and female trunks; so that leaflets are opposite in a male trunk and are located across from each other in two sides of the leaf rachis, whereas, leaflets in the female trunk are alternate on the leaf rachis (Figure 1).

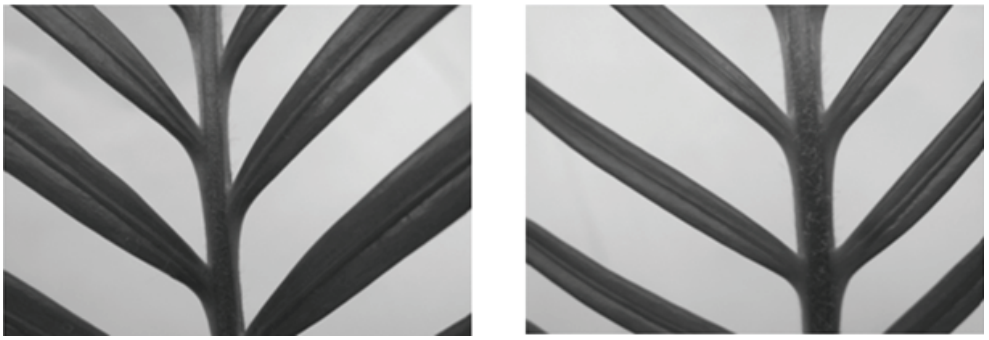

Figure 1. Arrangement of alternate in leaflets of female trunk (left), and opposite in leaflets of male trunk (right) of *Cycas revoluta*.

In the final one third of the leaf rachis, leaflets are compact and close together where the arrangement of the leaflets is irregular.

Results obtained in our work suggest that forming new leaflets at the male trunk starts after differentiation of previous leaflet; so that the leaf apex of leaf has differentiated and individual leaflets (Figure 2).

On the female trunks, initiation and formation of the new leaflets occurs before full differentiation of earlier leaflets, while the prior leaflets are not reach to full development and separate from it. Consequently, the apical leaf rachis in the female trunks of *Cycas revoluta* have two leaflets with one being more differentiated than another (Figure 2).

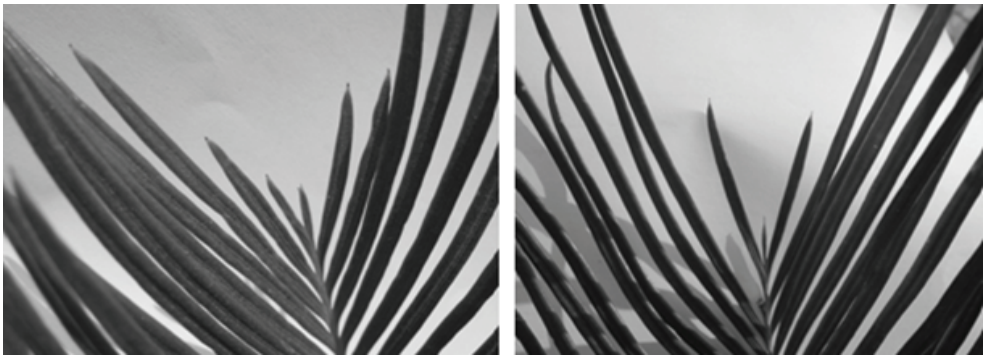

Figure 2. Formation of new leaflets in female trunk (left), and male trunk (right) of *Cycas revoluta*.

The position of the leaflet blades relative to the rachis occurs in three forms;

1. At the beginning of rachis and near the junction of off-shoots, leaflet blades are perpendicular to the leaf rachis and are almost horizontal to the ground surface (Figure 3).
2. In the middle part of the leaf, leaflet blades as the first case was not perpendicular to the rachis and located in angle of about 30° with respect to the leaf rachis and is not horizontal with respect to the earth's surface (Figure 3) and
3. In the terminal part of leaf rachis, the area of leaflet blades is aligned with the rachis and is at the same level (Figure 3).

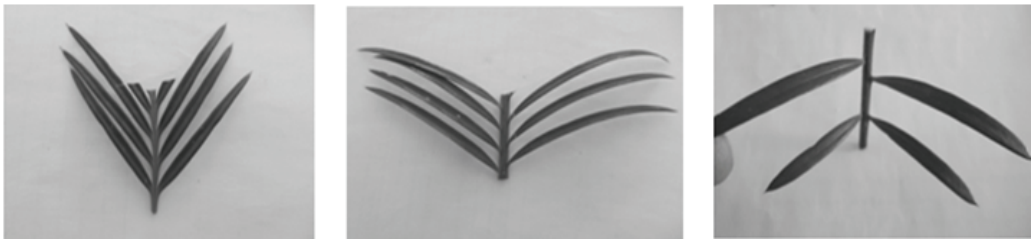

Figure 3. Arrangement of leaflets in base (right), center (middle) and top (left) of leaf in *Cycas revoluta*.

Roots in *Cycas revoluta* are white, creamy white, fleshy and dichotomous with primary and secondary roots which do not tend to move into deep soil (Stevenson, 1990). Size, diameter and root volume were very variable in *Cycas* and vary from very little branches and fleshy to lots of branches and subsidiary (Hill, 2012).
The study of morphological characteristics of male and female trunks of *Cycas revoluta* in the present study showed some differences.
In the female trunks, root volume is high and root has high subsidiary branches and its color is light brown (Figure 4).
In the male trunk, root volume compared to the female trunk is low with fewer branches, however is fleshy and has a tendency to bifurcate. The root color in male cones is cream and has a lighter color than the female trunk (Figure 4).

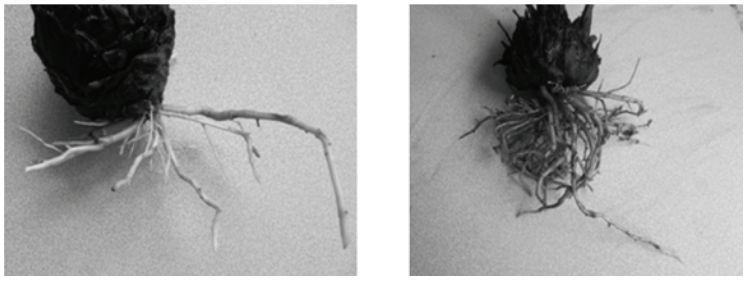

Figure 4. Root in male trunk (left), and female trunk (right) of *Cycas revoluta*

Quantitative data and analysis of variance for anatomical characteristics obtained from male and female trunks of *Cycas revoluta* that are shown in Tables 3, 4. Anatomical comparison of the leaf rachis showed that the number of cortex parenchyma layers is 6-10 and 7-9 in the male and female trunks, respectively (Table 5).
The lowest and highest layers were observed in male trunk. Stele diameter in male and female trunks is variable from 17.00 to 21.50 and 15.00 to 26.00 micrometers, respectively. Stele was not integrated and each strip is called a vascular bundle.
The leaf rachis of male and female trunks of *Cycas revoluta* has 1 to 2 procambium layers located between the central vascular bundles. In this taxon, the first procambium creates primary xylem and phloem and then produces the vascular cambium (Ladder, 1995).
The rachis has collenchyma bundles under the epidermis that offer sufficient solidity; so that the leaf rachis is like the semi-woody stems of other plants. Mesophyll contains parenchyma cells with spongy and palisade arrangement and has intercellular spaces.
This finding is consistent with Ladder (1995). Vascular bundles in the male and female trunks are equal (three bundles) and central. The diameter of pericycle in the male and female trunks are 3-5 and 3-4.5 micrometers, respectively. Pericycle surrounds the vascular bundles (Table 5).

Table 4. Discriminate Analysis (DA) of means of groups for anatomical characteristics of male and female trunks of *Cycas revoluta*.

Source of variances	Vilex λ	F	df_1	df_2	Sig.
No. of parenchyma layers in main axils of leaf (X_1)	0.97	0.21	1	10	0.65^{ns}
Stele diameter in main axils of leaf (X_2)	0.80	2.39	1	10	0.15^{ns}
No. of procambium layers in main axils of leaf (X_3)	1.00	0.00	1	10	0.10^{ns}
No. of vascular bundle in main axils of leaf (X_4)	a	-	1	10	-
Pericycle diameter in main axils of leaf (X_5)	0.87	1.81	1	10	0.20^{ns}
No. of trichoms in main axils of leaf (X_6)	0.98	0.17	1	10	0.68^{ns}
No. of upper parenchyma layers in leaflet (X_7)	0.89	1.21	1	10	0.29^{ns}
No. of lower parenchyma layers in leaflet (X_8)	0.96	0.38	1	10	0.54^{ns}
No. of stomata in upper surfaces of leaflet (X_9)	0.81	2.28	1	10	0.16^{ns}
No. of stomata in lower surfaces of leaflet (X_{10})	0.92	0.78	1	10	0.39^{ns}
Length of upper stomata in leaflet (X_{11})	0.92	0.86	1	10	0.37^{ns}
Length of lower stomata in leaflet (X_{12})	0.64	5.41	1	10	0.04^{*}
No. of trichoms in upper surface of leaflet (X_{13})	0.71	4.06	1	10	0.07^{ns}
No. of trichoms in upper surface of leaflet (X_{14})	0.95	0.46	1	10	0.51^{ns}
No. of cortex parenchyma layers in root (X_{15})	0.88	1.28	1	10	0.28^{ns}
No. of cambium layers in root (X_{16})	0.97	0.29	1	10	0.59^{ns}
Stele diameter in root (X_{17})	0.96	0.41	1	10	0.53^{ns}
Pericycle diameter in root (X_{18})	0.97	0.22	1	10	0.64^{ns}
No. of vascular bundle (X_{19})	-	-	1	10	-

*Significant at α = 5%, ^{ns}=Not significant

Table 5. Anatomical characters in main axis of leaf of male and female trunks of *Cycas revoluta*

Trunk / Anatomical characters	Female	Male
No. of cortex parenchyma layers	7-9 ± 2 (8)	6-10 ± 4 (8)
Stele diameter (μM)	15-26 ± 11 (20.5)	17-21.5 ± 5.4 (19.25)
No. of procambium layers	1-2 ± 1 (1.5)	1-2 ± 1 (1.5)
No. of vascular bundle	3	3
Pericycle diameter (μM)	3-4.5 ± 1.5 (3.75)	3-5 ± 2 (4)
No. of trichoms/mm^2	10-13 ± 3 (11.5)	8-15 ± 7 (11.5)
Type of trichoms	Protective	Protective

Leaf rachis epidermis in the male and female trunks of *Cycas revoluta* is covered with trichoms. Trichoms are protective, needle-shaped and multi-cellular and similar in the male and female trunks (Figure 5).

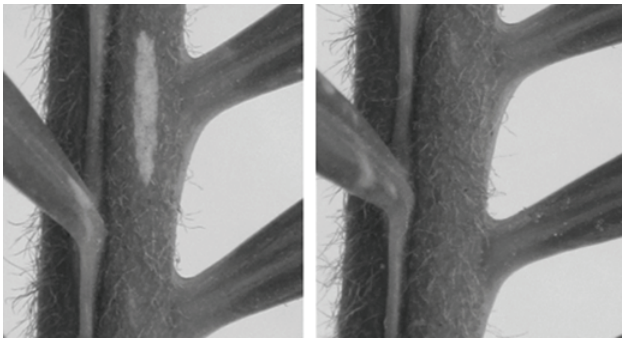

Figure 5. Trichoms on main axils of leaf in female trunk (left), and male trunk (right) of *Cycas revoluta*

The number of hairs in the rachis of the male and female trunks was counted as 8-15 and 10-13/mm^2, respectively. The presence of trichoms in the leaf rachis represents the adjustment of *Cycas* with the present climatic condition (Fan, 1990).

The anatomical study of leaflet in male and female trunks shows similarities and differences (Table 6).

Table 6. Anatomical characters in leaflets of male and female trunks of *Cycas revoluta*

Trunk / Anatomical characters	Female	Male
No. of upper parenchyma layers	8-9 ± 1 (8.5)	8-9 ± 1 (8.5)
No. of lower parenchyma layers	7-8 ± 1 (7.5)	7-8 ± 1 (7.5)
Stoma composition	Projecting	Projecting
Type of stoma	Actinocytic	Actinocytic
No. of upper stomata/mm^2	25-27 ± 2 (26)	24-30 ± 6 (27)
No. of lower stomata/mm^2	29-35 ± 6 (32)	26-37 ± 11 (31.5)
Length of upper stomata (μM)	5.5-8 ± 2.5 (6.75)	5-9.5 ± 4.5 (7.25)
Length of lower stomata (μM)	7-9.5 ± 2.5 (8)	6.5-8.5 ± 2 (7.5)
Type of trichoms	Protective and simple	Protective and simple
No. of trichoms in upper surface/mm^2	3-5 ± 2 (4)	3-8 ± 5 (5.5)
No. of trichoms in lower surface	5-10 ± 5 (7.5)	7-9 ± 2 (8)

Upper parenchyma in the male and female trunks have 7-9 and 8-9 layers, respectively. Parenchyma cells can be seen as a void and polygons. The number of lower parenchyma in the male and female trunks is similar and variable from 7 to 9 layers. Like angiosperms, mesophyll of *Cycas* leaflet includes palisade and spongy parenchyma. Primary xylem in middle veins is a primitive type. Secondary xylem is created from the cambium that is located between the two types of vascular tissue. The middle vein is surrounded by endodermis. Stoma type in the male and female trunks is alike. That is projecting and actinocytic; so that, with a cycle of subsidiary cell has been isolated from the epidermal cells. Subsidiary cells surround stoma by circular (Figure 6 and 7). *Cycas* leaflet is leathery and hard and epidermal cells have thickness wall and cuticle, and stomata in the lower leaf surface is depth (Ladder, 1995).

Upper stomata density of leaflet in female and male trunks ranges between 25 and 28, also 24 and 30/mm^2, respectively. Lower stomata density of leaflet in female and male trunks ranges between 29 and 35, as well 26 and 37/mm^2, respectively (Figure 6).

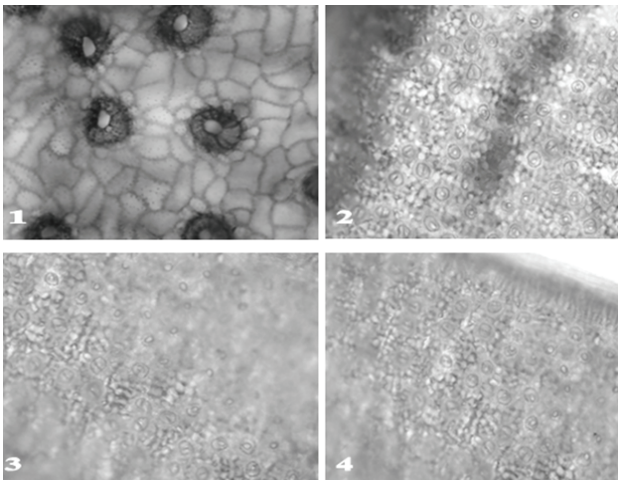

Figure 6. Stomata in epidermis of leaflets of *Cycas revoluta*, 1. Lower epidermis of female trunk, 2. Lower epidermis of male trunk, 3. Upper epidermis of female trunk, and 4. Upper epidermis of male trunk

Upper stomata length in female and male trunks is variable from 5.5 to 8.0 and 5.0 to 9.5 µM. Also, lower stomata length in female and male trunks is variable from 7.0 to 9.5 and 6.5 to 8.5 µM. Stomata density on each plant species was almost constant and its change is limited. Hair density in the lower surface of leaflets is higher than the upper surface; so that in the male and female trunks ranges between 5 and 10, also 7 and 9/mm^2 (Figure 7).

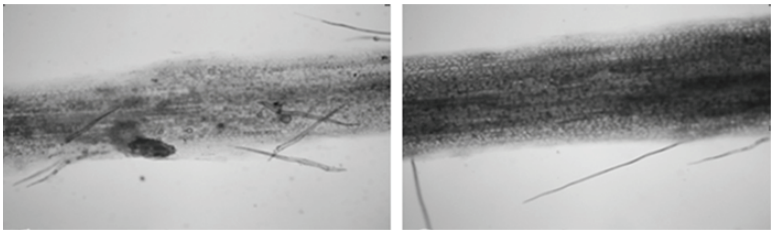

Figure 7. Trichoms on epidermis surface of female leaflet (left) and male leaflet (right) of *Cycas revoluta*

The study on anatomical characteristics of the root showed similarities and differences. Root parenchyma layers at male and female trunks are equal and including 8-9 layers. In transverse section of root, the number of cambium layers was 2-3 and in male and female is equal. The stele diameter in the female and male trunks ranges from 25 to 30 and 25 to 28 µM, respectively.

Table 7. Anatomical features of male and female roots in *Cycas revoluta*

Trunk	No. of cortex parenchyma layers	No. of cambium layers	Stele diameter (µM)	Pericycle diameter (µM)	No. of vascular bundles
Female	8-9 ± 1 (8.5)	2-3 ± 1 (1.5)	25-30 ± 5 (27.5)	0.50-0.55 ± 0.05 (0.525)	1
Male	8-9 ± 1 (8.5)	2-3 ± 1 (1.5)	25-28 ± 3 (26.5)	0.45-0.58 ± 0.13 (0.515)	1

Pericycle layer thickness in female and male trunks ranges from 0.50 to 0.55 and 0.45 to 0.58 µM, respectively. Current study revealed that the vascular bundles in male and female trunks are equal. The studies on cycads root showed that there are four types of root as follows; primary root, secondary or lateral root, swollen roots and coralloid root (Stevenson, 1990). The results of the present study are consistent with these results.

CONCLUSIONS

Sex determination of cycad (*Cycas revoluta* Thunb.) before maturity reports here.
Anatomical and morphological studies showed that there are some differences between male and female trunks.

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