# Leaf Anatomy in Cross-section Features of 29 Genotypes of Oil Tea from Hainan Island and Their Systematic Significance

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【Summary】

Oil tea belongs to the *Camellia* genus, which is mainly distributed in southern China and is one of the four woody edible oil plants in the world. Recently, we identified some genotypes of oil-tea plants on Hainan Island. However, we did not know whether these plants belonged to *C. oleifera*. This study compared 11 leaf anatomical characters for 29 genotypes of oil tea from Hainan Island to 1 genotype of *C. oleifera* from Hunan Province, China. A principal component analysis and cluster analysis were conducted using transformed data for quantitative and qualitative characters of the leaf anatomy to explain their relationships. Results indicated that leaf thickness ranged 230.45~460.79 μm. Diameters of the midrib vessels ranged 349.63~729.85 μm. Palisade tissues consisted of 1 or 3 layers of arranged columnar cells, accounting for 25.10 or 41.47%, respectively, of the leaf thickness. Similarly, spongy tissues accounted for 42.14~72.49% of the thickness of the leaves. The area of the adaxial epidermis ranged 384.75~1643.90 $μm^2$, and the perimeter of the adaxial epidermal cells varied from 55.74 to 183.05 μm. Leaves classified according to genotype were divided into 2 groups using the principal component and cluster analyses. We observed differences in leaf anatomical characters of genotypes collected between the 2 regions, suggesting that oil tea from Hainan Island has a close relative relationship with *C. oleifera* and belongs to *Camellia* sect. *Oleifera*. Leaf morphological characteristics could provide important information for genotype identification of these oil-tea plants in the future.

**Key words:** *Camellia* Sect. Oleifera, leaf anatomy, principal component analysis, cluster analysis.

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研究報告

# 海南島29份油茶基因型葉片解剖及其系統學意義

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## 摘　要

　　油茶屬山茶屬植物，是世界四大木本食用油料植物之壹，主要分佈在中國南方。近年來，我們對海南島油茶進行了基因型鑒定。然而，我們不知道這些植物是否屬於普通油茶。本研究比較了海南島29份油茶基因型與1份普通油茶基因型的11個葉解剖特徵。利用葉片解剖學定量和定性性狀的轉換數據進行主成分分析和聚類分析，以解釋二者之間的關系。結果表明：葉厚230.45~460.79 μm，中脈導管直徑349.63~729.85 μm，柵欄組織由1~3層排列整齊的柱狀細胞組成，分別占葉厚的25.10~41.47%。同樣，海綿狀組織占葉片厚度的42.14~72.49%。近軸葉面積384.75~1643.90 μm²，近軸面細胞周長55.74~183.05 μm。根據基因型劃分，利用主成分分析和聚類分析法將30份分為兩組。我們觀察了兩個地區不同基因型葉片解剖特徵的差異，認為海南島油茶與普通油茶有著密切的親緣關系，屬於油茶組。油茶葉片形態特徵可為今後油茶的基因型鑒定提供重要資訊。

**關鍵詞**：油茶組、葉片解剖、主成分分析、聚類分析。

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## INTRODUCTION

The genus *Camellia* includes many oil-tea plant species which are widely cultivated for their seeds which contain edible oil (Mondal 2002). Oil tea is distributed across 15 provinces in southern China, including Hunan, Jiangxi, Guangxi, Zhejiang, Fujian, and Guangdong Provinces (He et al. 2002, Xiong et al. 2019a). Hainan Island is one of the largest islands in China, and its biodiversity is ecologically significant (Xu et al. 2020). In recent years, some specimens of subtropical oil-tea trees were found on Hainan Island (Yang et al. 2012). According to Zheng et al. (2016), the unique climate and environmental conditions of Hainan Island have fostered a rich and distinctive oil-tea population. Wang et al. (2017) studied the growth of 1-yr-old seedlings, while Wang et al. (2016) examined the economic charac-teristics of fruit of oil tea on Hainan Island. Both suggested that these plants belonged to *C. oleifera*. However, it had large, brown fruit, so it could be distinguished from *C. oleifera*. It is not yet clear whether these oil-tea trees originating in the Hainan area belong to *C. oleifera*. Those studies failed to examine or answer taxonomic aspects of oil-tea plants from Hainan Island.

Leaf anatomy has always played a crucial role in plant taxonomy and plant classification. For example, the hypodermis, crystals (Heintzelman and Howard 1948), trichomes, stomata, the cuticle (Stace 1965), and stone cells (Baranova 1972) proved to be critical in taxonomic classification. Although the leaf is the 1 plant organ that differs the most anatomically based on the hierarchical level of species, genus, and family, and despite the

influence of environmental factors on leaf morphology, many anatomical characters are still useful in systematic studies (Huseyin and Melahat 2011). In the sect. *Camellia*, previous work used leaf anatomy to reveal relationships among taxa (Lin et al. 2008, Pi et al. 2009, Zheng et al., 2013). Lin et al. (2008) investigated the leaf anatomy of 21 species representing sect. *Oleifera* H. T. Chang and sect. *Paracamellia* Sealy in the genus *Camellia* using light microscopy. Based on 17 anatomical and cytological characters of leaves, Lin et al. classified sect. *Oleifera* and sect. *Paracamellia* into 2 different groups. Pi et al. (2009) examined leaf morphological and anatomical characters of 54 species and 3 varieties in *Camellia* sect. *Camellia*. Zheng et al. (2013) also observed anatomical characteristics of leaf and midrib transections of 61 cultivars of *C. oleifera* from Zhejiang Province, China and demonstrated relationships among the 61 cultivars using a cluster analysis. These studies showed that leaf characteristics are powerful tools for separating and identifying plants in sect. *Camellia*.

Although descriptions of anatomical characteristics and relational analyses of leaf transections in *Camellia* do exist, differences in these traits among the various oil-tea genotypes are still unclear, especially for the new oil-tea genotypes from Hainan Island. Clearly, more work is needed to clarify the taxonomy of sect. *Camellia*. Therefore, the purpose of our study was to enrich our knowledge of leaf anatomy and provide a basis for further investigations of systematic classification using data of leaf anatomy in sect. *Camellia*.

## MATERIALS AND METHODS

Twenty-nine oil-tea genotypes collected from Hainan Island and 1 genotype of *C. oleifera* from Hunan Province were used in this study as experimental materials (Table 1). Samples from Hainan Island were collected from Chengmai, Tunchang, Qiongzhong, and Danzhou Counties. The 1 sample from Hunan Province was harvested from an oil-tea nursery at the Central South University of Forestry and Technology (Changsha, China).

Standard micro-technical methods for anatomy as described by Cutler (1978) were used to prepare transverse sections. Leaves were collected from each genotype and cut into pieces. Samples were taken from the middle part of a leaf and were fixed in a formaldehyde-acetic acid-alcohol (FAA) solution (commercial formalin, glacial acetic acid, and 70% ethanol in a ratio of 5:5:90 v/v, respectively) (Stern and Judd 2002). Samples were dehydrated and embedded in paraffin, stained with 0.5% safranin and fast green, and mounted in neutral resin (Xiong et al. 2019b). Samples were examined at 100× using a BX-51 microscope (Olympus, Tokyo, Japan). Specimens were also photographed with the BX-51 Olympus microscope.

Eleven leaf characters were selected and used in a cluster analysis and principle component (PC) analysis (PCA). These characters included the area of adaxial epidermal cells (AAD), perimeter of adaxial epidermal cells (PAD), area of abaxial epidermal cells (AAB), perimeter of abaxial epidermal cells (PAB), thickness of the leaf (TL), the ratio of adaxial epidermal cells to the leaf thickness (ADL), the ratio of abaxial epidermal cells to the leaf thickness (ABL), the ratio of the palisade parenchyma to the leaf thickness (PPL), the ratio of the spongy parenchyma to the leaf thickness (SPL), the ratio between of the vessel in the midrib to the leaf thickness (PML), and number of layers of palisade tissues (LP).

All average values for the PCA were transformed using Eq. 1 according to Pi et al. (2009):

$$D_i = \frac{n\overline{X_i}}{\sum\limits_{i=1}^{n} \overline{X_i}}$$ ..............................................(Eq. 1)

where $D_i$ is the transformed value, $X_i$ is the average value of character $i$, and $n$ is the number of genotypes examined. $D_i$ makes it possible to compare data calculated in various units. The PCA and similarity analyses were conducted using SPSS software (Geraci et al. 2012, Xiong et al. 2020). Taxonomic relationships were analyzed using a dendrogram based on a data matrix of taxonomic distances

calculated using the unweighted pair group method with an arithmetic mean analysis (Lee et al. 2008). Figures depicting these analyses were drawn using Origin software (vers. 8.5; Origin Laboratory, Northampton, MA, USA).

## RESULTS

### Characters in the transverse section

Eleven leaf characters in the transverse section are presented in Table 2. All leaves

**Table 1. Source of material of 30 genotypes of oil tea**

| No. | Origin (County, Town) | Code | Longitude (E) | Latitude (N) |
|---|---|---|---|---|
| 1 | Chengmai, Jinjiang | CMBF-61 | 109°50'61.4" | 19°39'12.3" |
| 2 | Chengmai, Fushan | CMFS-63 | 109°58'50.3" | 19°53'13.5' |
| 3 | Chengmai, Fushan | CMFS-64 | 109°58'50.6" | 19°53'14.8'' |
| 4 | Chengmai, Fushan | CMFS-65 | 109°58'50.1" | 19°53'15.8" |
| 5 | Chengmai, Fushan | CMFS-66 | 109°58'50.1" | 19°53'15.8" |
| 6 | Chengmai, Honggangnongchang | CMHGNC-62 | 110°3'12" | 19°34'0" |
| 7 | Chengmai, Jaile | CMJL-6 | 109°58'50.1" | 19°53'15.8" |
| 8 | Chengmai, Jaile | CMJL-62 | 110°0'93.4" | 19°38'42.3" |
| 9 | Chengmai, Jaile | CMJLCL-61 | 109°55'21.1" | 19°40'10.8" |
| 10 | Chengmai, Jaile | CMJLCL-62 | 109°55'21.1" | 19°40'10.8" |
| 11 | Chengmai, Jaile | CMJLCL-63 | 109°58'52.5" | 19°37'08.7'' |
| 12 | Chengmai, Jaile | CMJLLX-61 | 109°59'35" | 19°32'12" |
| 13 | Chengmai, Zhongxin | CMSK-62 | 109°56'37" | 19°38'27" |
| 14 | Chengmai, Jinjiang | CMTP-61 | 110°0'6.6" | 19°38'54.5" |
| 15 | Chengmai, Jinjiang | CMTP-62 | 110°0'7.6" | 19°38'55.9" |
| 16 | Chengmai, Jiale | CMZLLX-61 | 109°59'35" | 19°32'12" |
| 17 | Chengmai, Zhongxing | CMZX-61 | 109°55'39" | 19°39'57" |
| 18 | Chengmai, Zhongxing | CMZX-62 | 109°55'39" | 19°39'57" |
| 19 | Chengmai, Zhongxing | CMZX-65 | 109°56'58.8" | 19°37'32.5" |
| 20 | Chengmai, Zhongxing | CMZX-66 | 109°56'58.8" | 19°37'32.5" |
| 21 | Chengmai, Zhongxing | CMZX-67 | 109°56'58.8" | 19°37'32.5" |
| 22 | Chengmai, Zhongxing | CMZX-68 | 109°56'51.7" | 19°37'10.8" |
| 23 | Danzhou, Heqing | DZHQ-61 | 109°43'46.2" | 19°31'35.5'' |
| 24 | Qiongzhong, Changzhen | QZCZ-4 | 109°52'55.6" | 18°56'53.1" |
| 25 | Qiongzhong, Changzhen | QZCZ-9 | 109°52'55.6" | 18°56'53.1" |
| 26 | Qiongzhong, Changzhen | QZWZ-62 | 109°54'15." | 19°10'11.4" |
| 27 | Qiongzhong, Changzhen | QZWZ-64 | 109°54'15." | 19°10'11.4" |
| 28 | Tunchang, Nankun | TCNK-63 | 109°55'52.1" | 19°20'12" |
| 29 | Tunchang, Nankun | TCNK-64 | 109°59'03.1" | 19°19'38.6" |
| 30 | Changsha, Tianxin | Yongxing7608 | 112°59' 40" | 28° 7' 56" |

**Table 2. Characters in transverse section of 30 genotypes of oil tea**

| No. | Upper epidermis | | | Lower epidermis | | | Palisade tissue thickness | Spongy tissue thickness | Vessel in midrib thickness | Leaf thickness |
|---|---|---|---|---|---|---|---|---|---|---|
| | Area (µm²) | Perimeter (µm) | Thickness (µm) | Area (µm²) | Perimeter (µm) | Thickness (µm) | (µm) | (µm) | (µm) | (µm) |
| 01 | 500.42±220.05 | 86.62±17.56 | 26.55±3.89 | 478.55±220.98 | 91.50±19.37 | 17.66±2.15 | 82.99±7.270 | 132.73±13.62 | 478.76±11.33 | 279.96±13.55 |
| 02 | 684.21±295.63 | 124.60±32.43 | 18.40±2.51 | 389.11±183.05 | 95.10±24.82 | 13.44±2.16 | 87.12±8.330 | 164.25±18.21 | 530.71±7.54 | 309.83±11.53 |
| 03 | 550.63±194.66 | 105.80±17.80 | 19.33±3.28 | 532.74±221.90 | 106.37±32.21 | 13.94±2.96 | 71.90±9.010 | 169.58±7.23 | 367.86±2.45 | 284.59±5.530 |
| 04 | 1643.90±470.84 | 183.05±37.04 | 29.48±9.79 | 609.18±265.24 | 120.60±48.04 | 14.83±2.45 | 107.64±26.76 | 264.58±11.75 | 674.90±7.95 | 365.01±11.02 |
| 05 | 561.29±231.48 | 116.97±23.38 | 35.69±4.30 | 312.87±131.34 | 88.91±23.27 | 20.54±1.88 | 149.60±19.68 | 205.87±8.96 | 685.93±3.35 | 449.71±21.88 |
| 06 | 1147.24±428.74 | 142.31±29.11 | 38.37±6.60 | 568.98±217.29 | 99.50±24.52 | 21.53±5.69 | 102.60±8.90 | 152.73±12.72 | 448.74±9.99 | 333.43±13.79 |
| 07 | 497.13±200.45 | 92.97±22.18 | 21.07±2.09 | 479.69±286.55 | 109.84±32.67 | 17.75±3.30 | 75.04±7.05 | 175.15±12.64 | 729.85±1.98 | 272.84±14.33 |
| 08 | 582.91±347.35 | 91.48±23.90 | 25.70±5.90 | 449.26±140.21 | 75.11±18.43 | 17.53±2.16 | 75.29±5.08 | 169.88±4.56 | 573.85±15.12 | 290.13±5.04 |
| 09 | 524.96±274.08 | 105.99±25.77 | 24.57±7.25 | 653.21±206.69 | 102.12±18.48 | 19.13±3.11 | 69.93±14.05 | 158.38±11.73 | 549.94±3.86 | 274.35±11.98 |
| 10 | 539.19±204.59 | 105.17±22.76 | 25.72±3.71 | 454.41±147.06 | 85.05±22.15 | 16.73±3.92 | 113.81±22.04 | 148.65±11.80 | 462.78±4.70 | 309.56±4.75 |
| 11 | 967.57±327.11 | 112.84±18.31 | 35.14±8.69 | 595.03±182.28 | 103.18±26.88 | 19.23±2.26 | 110.86±17.82 | 159.77±8.61 | 445.44±5.45 | 327.62±6.44 |
| 12 | 951.21±369.78 | 146.59±25.99 | 50.72±8.06 | 324.52±149.58 | 108.76±20.46 | 24.66±3.37 | 104.80±12.08 | 232.05±16.23 | 724.21±10.29 | 411.78±10.09 |
| 13 | 476.55±377.13 | 88.63±24.24 | 27.90±6.70 | 279.42±116.09 | 86.75±28.28 | 16.02±3.91 | 108.05±7.93 | 116.03±11.55 | 378.17±1.54 | 272.28±3.34 |
| 14 | 793.86±508.75 | 107.01±32.67 | 33.29±9.39 | 362.09±143.62 | 253.54±44.21 | 15.63±2.73 | 77.40±7.90 | 156.28±7.29 | 349.63±8.70 | 266.81±5.51 |
| 15 | 609.89±388.87 | 99.25±35.26 | 33.15±5.15 | 409.44±209.92 | 83.47±16.74 | 23.13±5.03 | 86.05±8.78 | 198.60±12.94 | 577.97±7.03 | 331.78±7.13 |
| 16 | 570.46±253.05 | 99.86±23.30 | 27.45±6.13 | 309.25±127.85 | 81.55±19.48 | 13.95±2.33 | 86.90±14.17 | 139.08±12.11 | 387.30±3.47 | 271.45±10.92 |
| 17 | 709.41±504.26 | 113.07±36.54 | 36.19±7.07 | 472.72±219.46 | 87.22±28.54 | 22.95±4.06 | 101.10±12.08 | 195.99±10.52 | 494.40±2.61 | 361.16±6.45 |
| 18 | 411.48±142.49 | 104.56±31.42 | 30.84±9.21 | 371.30±163.10 | 89.33±20.59 | 18.16±3.78 | 75.31±9.50 | 173.04±15.92 | 389.78±3.16 | 299.99±10.57 |
| 19 | 520.48±229.01 | 98.18±29.83 | 29.37±3.03 | 410.83±100.99 | 90.18±12.96 | 22.26±4.06 | 130.34±11.84 | 132.43±7.16 | 464.97±1.82 | 314.27±5.15 |
| 20 | 374.35±392.61 | 55.74±45.22 | 33.93±4.15 | 363.19±83.29 | 72.52±18.17 | 20.16±2.75 | 77.48±9.53 | 194.64±10.10 | 357.00±2.41 | 327.69±9.48 |
| 21 | 637.89±319.69 | 118.46±21.78 | 48.39±3.69 | 524.91±163.69 | 95.79±24.12 | 28.14±3.87 | 110.52±8.84 | 241.43±22.99 | 483.16±3.02 | 428.24±20.61 |
| 22 | 384.75±132.49 | 93.62±24.31 | 20.03±4.82 | 438.99±136.17 | 82.33±19.46 | 14.75±2.93 | 68.47±12.66 | 132.02±6.86 | 463.11±10.09 | 230.45±10.43 |
| 23 | 876.30±538.95 | 121.56±38.46 | 36.27±8.95 | 795.50±364.35 | 114.20±25.89 | 25.66±3.90 | 105.53±10.47 | 226.99±21.98 | 636.68±10.31 | 378.11±8.57 |
| 24 | 749.41±385.91 | 110.09±29.01 | 35.08±5.31 | 406.83±130.08 | 80.32±14.44 | 17.52±2.27 | 84.22±9.36 | 160.25±9.18 | 505.76±3.81 | 300.99±5.44 |
| 25 | 698.43±197.70 | 123.93±27.75 | 24.77±3.52 | 511.00±176.24 | 81.50±18.24 | 18.71±2.59 | 92.85±13.22 | 163.42±10.88 | 525.85±1.83 | 305.61±5.89 |
| 26 | 760.16±284.80 | 104.08±17.83 | 26.93±3.51 | 756.75±266.95 | 112.82±23.23 | 22.93±2.72 | 94.32±8.14 | 185.42±12.98 | 383.07±4.06 | 341.67±3.94 |
| 27 | 707.04±171.07 | 108.69±17.35 | 27.91±3.80 | 466.78±192.77 | 89.65±20.23 | 21.78±2.71 | 96.24±9.38 | 159.09±13.82 | 431.17±4.25 | 304.64±13.23 |
| 28 | 747.57±539.11 | 111.08±31.17 | 25.29±4.54 | 439.46±161.34 | 95.73±19.86 | 16.17±2.06 | 94.60±22.59 | 150.50±24.82 | 502.51±5.01 | 301.29±7.150 |
| 29 | 598.96±318.71 | 97.65±26.54 | 17.03±2.05 | 482.63±151.73 | 101.85±20.44 | 15.49±4.29 | 78.61±7.42 | 159.96±25.35 | 537.39±7.40 | 269.35±18.84 |
| 30 | 459.14±116.75 | 91.99±13.56 | 28.52±2.81 | 695.22±225.20 | 101.22±21.02 | 21.93±3.04 | 128.2±9.49 | 277.65±40.67 | 603.19±8.17 | 460.79±12.30 |

were bifacial and were composed of wax, epidermis, palisade parenchyma, spongy parenchyma, and vascular tissues (Figs. 1, 2). One of the most pronounced differences observed was in leaf thickness (Table 2, Fig. 1).

Leaf thickness ranged from 230.45 (Fig. 2G) to 460.79 μm (Fig. 2O), and most genotypes ranged 200~400 μm; however, a few genotypes had a leaf thickness exceeding 400 μm (Figs. 1E, L, 2F, O). Diameters of midrib ves-
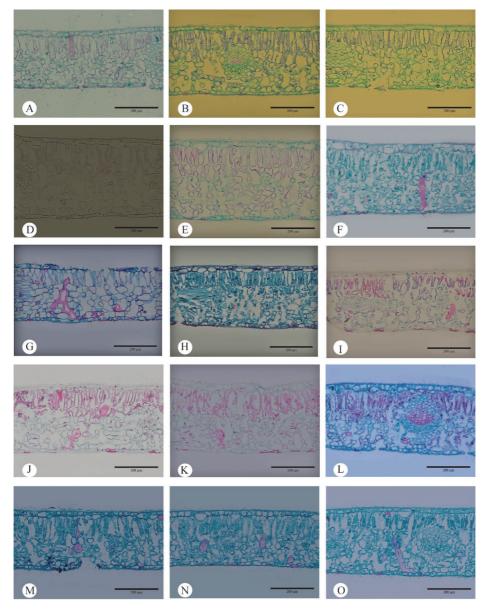


**Fig. 1. Transverse section of oil-tea leaves from Hainan Island. A, CMBF-61; B, CMFS-63; C, CMFS-64; D, CMFS-65; E, CMFS-66; F, CMHGNC-62; G, CMJL-6; H, CMJL-62; I, CMJLCL-61; J, CMJLCL-62; K, CMJLCL-63; L, CMJLLX-61; M, CMSK-62; N, CMTP-61; O, CMTP-62.**

sels in the 30 genotypes ranged from 349.63 (Fig. 2A) to 729.85 μm (Fig. 1G), and were 1.09~2.67-times larger than the leaf thickness. All examined genotypes had clearly developed mesophyll tissues. The mesophyll consisted of palisade and spongy tissues. Palisade tissues of examined leaves consisted of 1 or 3 layers of arranged columnar cells (Figs. 1, 2), and the percent of palisade tissues in leaf thickness accounted for 25.10~41.47% (Table 2). Of these 30 genotypes, all 29 Hainan genotypes had leaves with a single layer of palisade tissue (Figs. 1, 2). However, leaves from Hunan Province had 3 layers of palisade tissue (Fig. 2O). Spongy tissues accounted for a large proportion of the leaf thickness and ranged 42.14~72.49% (Table 2). All genotypes had irregular stone cells in the spongy tissue (Figs. 1, 2), but a few had columnar stone cells through the palisade and spongy tissues (Figs. 1G, 2B, 2M).

Large variations in the perimeter and areas of adaxial and abaxial epidermal cells were observed among genotypes. The area of adaxial epidermal cells varied substantially among the 30 genotypes, ranging from 384.75 μm$^2$ in Fig. 2G to 1643.90 μm$^2$ in Fig. 1D (Table 2). Among the 30 genotypes, the area of abaxial epidermal cells was generally smaller than that of adaxial epidermal cells, and the mean varied between 279.42 μm$^2$ in Fig. 1M and 795.50 μm2 in Fig. 2H (Table 2). The perimeter of the adaxial epidermal cells ranged from 55.74 μm in Fig. 2E to 183.05 μm in Fig. 1D, while the perimeter of abaxial epidermal cells varied from 72.52 μm in Fig. 2E to 253.54 μm in Fig. 2A (Table 2). The proportion of the leaf thickness accounted for by adaxial epidermal cells ranged from a low of 5.94% in Fig. 1B to a high of 12.48% in Fig. 2A, while the proportion of abaxial epidermal cells accounted for 4.06% in Fig. 1D and 7.15% in Fig. 2L (Table 2).

## PCA based on measurements of leaf transverse traits

Results of the PCA based on leaf transverse traits (Table 3) indicated that 60.06% of the cumulative variance was explained by 3 PCs. The PCA results indicated that the sum of components 1 and 2 accounted for 45.24% of the total variance, and PC1 and PC2 represented the thickness of the leaf and the area of abaxial epidermal cells, respectively. A scatterplot of PC1 and PC2 (Fig. 3) indicated that Hunan genotype no. 30 was distinct from most of Hainan genotypes (nos. 1~3, 5~8, 10, 12~22, 24, 25, and 27~29). A few Hainan genotypes, namely nos. 9, 11, 23, and 26 were close to Hunan genotype no. 30. However, Hainan genotype no. 4 was distinct from all the others, but was closer to most of the Hainan oil-tea genotypes than to the Hunan oil-tea genotype.

## Cluster analysis

The cluster analysis divided the 30 genotypes into two main clusters: cluster 1 (C1) consisted of the 29 genotypes native to the Hainan region, and cluster 2 (C2) consisted of only the *C. oleifera* genotype (no. 30) from Hunan Province (Fig. 4). Upon closer inspection, C1 contained 2 subclusters: subcluster 1 (SC1) consisted of 28 genotypes collected from Chengmai, Tunchang, Qiongzhong, and Danzhou Counties, and subcluster 2 (SC2) contained only one genotype (no. 4) collected from Chengmai County (Fig. 4).

## DISCUSSION

Leaf anatomy is very important in taxonomy and classification of *Camellia* species since leaf transverse characters are easily observed and can successfully be utilized as a classical source of data used in plant taxonomy (Brittan 1970, Lubke and Phipps 1973). Multivariate analyses including the PCA and
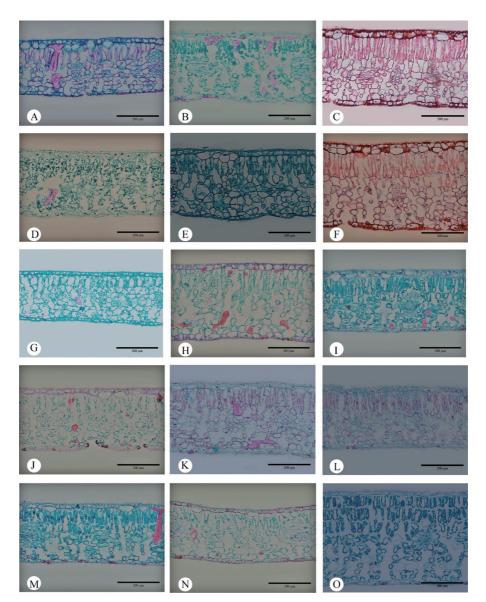
**Fig. 2. Transverse section of oil-tea leaves from Hainan Island and Hunan Province. A, CMZLLX-61; B, CMZX-61; C, CMZX-62; D, CMZX-65; E, CMZX-66; F, CMZX-67; G, CMZX-68; H, DZHQ-61; I, QZCZ-4; J, QZCZ-9; K, QZWZ-62; L, QZWZ-64; M, TCNK-63; N, TCNK-64; O, Yongxing7608.**

cluster analysis turned out to be very useful tools for differentiating plants (Kim et al. 2004, Giselle et al. 2018). Previous work described leaf transverse characters in the genus *Camellia* (Deng et al. 1992, Wang et al. 2007, Lin et al. 2008, Lu et al. 2008a). Deng et al. (1992) studied the anatomical structure of mature leaves for 15 major oil-tea *Camellia* spp., indicating that both the upper and lower foliar epidermis layers include only 1 layer of irregular, polygonal cells, while the palisade includes 1 or 3 layers of arranged colum-

**Table 3. Data matrix used in the principal component analysis (PCA) of 30 genotypes of oil tea**

| No. | AAD | PAD | AAB | PAB | TL | ADL | ABL | PPL | SPL | PML | LP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.742 | 0.789 | 1.001 | 0.919 | 1.001 | 1.030 | 1.058 | 1.003 | 0.867 | 1.079 | 1 |
| 2 | 1.014 | 1.135 | 0.814 | 0.955 | 0.814 | 0.639 | 0.722 | 0.952 | 0.969 | 1.081 | 1 |
| 3 | 0.816 | 0.964 | 1.114 | 1.069 | 1.114 | 0.737 | 0.823 | 0.857 | 1.090 | 0.816 | 1 |
| 4 | 2.437 | 1.668 | 1.274 | 1.212 | 1.274 | 0.878 | 0.688 | 0.999 | 1.326 | 1.167 | 1 |
| 5 | 0.832 | 1.066 | 0.654 | 0.893 | 0.654 | 0.856 | 0.772 | 1.128 | 0.838 | 0.962 | 2 |
| 6 | 1.701 | 1.297 | 1.190 | 1.000 | 1.190 | 1.246 | 1.091 | 1.043 | 0.838 | 0.849 | 1 |
| 7 | 0.737 | 0.847 | 1.003 | 1.104 | 1.003 | 0.835 | 1.091 | 0.931 | 1.174 | 1.688 | 1 |
| 8 | 0.864 | 0.833 | 0.940 | 0.755 | 0.940 | 0.965 | 1.007 | 0.877 | 1.072 | 1.248 | 1 |
| 9 | 0.778 | 0.966 | 1.366 | 1.026 | 1.366 | 0.975 | 1.175 | 0.864 | 1.055 | 1.265 | 2 |
| 10 | 0.799 | 0.958 | 0.950 | 0.854 | 0.950 | 0.900 | 0.907 | 1.246 | 0.878 | 0.943 | 2 |
| 11 | 1.434 | 1.028 | 1.245 | 1.037 | 1.245 | 1.160 | 0.990 | 1.145 | 0.893 | 0.858 | 2 |
| 12 | 1.410 | 1.336 | 0.679 | 1.093 | 0.679 | 1.333 | 1.007 | 0.860 | 1.032 | 1.110 | 1 |
| 13 | 0.706 | 0.808 | 0.584 | 0.872 | 0.584 | 1.105 | 0.990 | 1.345 | 0.779 | 0.876 | 1 |
| 14 | 0.846 | 0.910 | 0.647 | 0.819 | 0.647 | 1.095 | 0.856 | 1.084 | 0.936 | 0.900 | 1 |
| 15 | 0.610 | 0.953 | 0.777 | 0.897 | 0.777 | 1.116 | 1.024 | 0.850 | 1.055 | 0.820 | 1 |
| 16 | 1.177 | 0.975 | 0.757 | 2.547 | 0.757 | 1.355 | 0.990 | 0.982 | 1.072 | 0.827 | 1 |
| 17 | 0.904 | 0.904 | 0.856 | 0.839 | 0.856 | 1.084 | 1.175 | 0.877 | 1.096 | 1.099 | 1 |
| 18 | 1.052 | 1.030 | 0.989 | 0.876 | 0.989 | 1.084 | 1.074 | 0.948 | 0.993 | 0.864 | 2 |
| 19 | 0.772 | 0.895 | 0.859 | 0.906 | 0.859 | 1.008 | 1.192 | 1.406 | 0.770 | 0.934 | 1 |
| 20 | 0.555 | 0.790 | 0.760 | 0.729 | 0.760 | 1.127 | 1.041 | 0.799 | 1.086 | 0.687 | 1 |
| 21 | 0.946 | 1.079 | 1.098 | 0.962 | 1.098 | 1.225 | 1.108 | 0.874 | 1.032 | 0.712 | 1 |
| 22 | 0.570 | 0.853 | 0.918 | 0.827 | 0.918 | 0.943 | 1.074 | 1.006 | 1.048 | 1.268 | 1 |
| 23 | 1.299 | 1.107 | 1.664 | 1.147 | 1.664 | 1.040 | 1.142 | 0.945 | 1.097 | 1.063 | 1 |
| 24 | 1.111 | 1.003 | 0.851 | 0.807 | 0.851 | 1.268 | 0.974 | 0.948 | 0.973 | 1.060 | 1 |
| 25 | 1.035 | 1.129 | 1.069 | 0.819 | 1.069 | 0.878 | 1.024 | 1.030 | 0.979 | 1.086 | 1 |
| 26 | 1.127 | 0.948 | 1.583 | 1.134 | 1.583 | 0.856 | 1.125 | 0.935 | 0.993 | 0.707 | 1 |
| 27 | 1.048 | 0.990 | 0.976 | 0.901 | 0.976 | 0.997 | 1.192 | 1.070 | 0.955 | 0.893 | 1 |
| 28 | 1.108 | 1.012 | 0.919 | 0.962 | 0.919 | 0.910 | 0.907 | 1.064 | 0.915 | 1.053 | 1 |
| 29 | 0.888 | 0.890 | 1.009 | 1.023 | 1.009 | 0.683 | 0.974 | 0.989 | 1.086 | 1.259 | 1 |
| 30 | 0.681 | 0.838 | 1.454 | 1.017 | 1.454 | 0.672 | 0.806 | 0.942 | 1.103 | 0.826 | 3 |

AAD, area of adaxial epidermal cells; PAD, perimeter of adaxial epidermal cells; AAB, area of abaxial epidermal cells; PAB, perimeter of abaxial epidermal cells; TL, thickness of the leaf; ADL, ratio of adaxial epidermal cells to leaf thickness; ABL, ratio of abaxial epidermal cells to leaf thickness; PPL, ratio of palisade parenchyma to leaf thickness; SPL, ratio of spongy parenchyma to leaf thickness; PML, ratio of the vessel in the midrib to leaf thickness; LP, no. of layers of palisade tissues.

nar cells. They also reported within-species variations in *Camellia* leaf thicknesses, with a range of 205.3~471.4 μm. Our study also revealed that the 29 Hainan genotypes were similar in some respects of *Camellia* leaf anatomy, but leaf thickness varied among the genotypes. As shown in Table 2, the 1 genotype of *C. oleifera* native to China had the largest leaf thickness of 460.79 μm. Lu et al. (2008a) investigated a comparative anatomi-
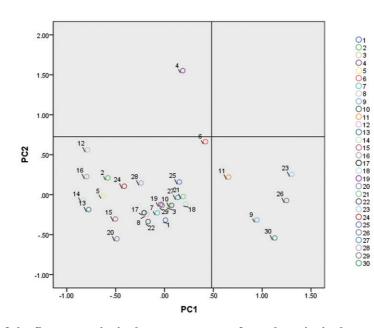
**Fig. 3. Plot of the first two principal component axes from the principal component analysis of leaf anatomy characteristics of 30 genotypes. Morphological codes correspond to those listed in Table 1.**

cal and Fourier transform infrared study on leaves of 18 disputed species of sect. *Tuberculata* (*Camellia*, Theaceae) and found that the thickness of the palisade parenchyma significantly varied within the same species distributed in various ecological regions. They stated that palisade parenchyma layers could be regarded as a character with systematic value at the species level. Of note, we found that the palisade parenchyma significantly varied in different regions. In our study, *Camellia* genotypes native to the Hainan area had 1 or 2 layers of palisade parenchyma cells, while those native to the Chinese mainland had 3 layers. This indicated that the palisade parenchyma layer is a reliable indicator. Zhang et al. (2013) examined the leaf anatomical structures of *C. oleifera* specimens in different ecotypes, and suggested significant differences in the thicknesses of the upper and lower epidermis, cutin layer, and stockade and spongy tissues. Liao et al. (2015) evaluat-

ed the anatomical characteristics of leaves of 5 *C. oleifera* 'Xianglin' series clones, indicating a significant difference in the palisade tissue/leaf thickness ratio. Similar results were observed in our study with the proportion of palisade tissue cells in leaves significantly varying among genotypes. Wang et al. (2007) compared the leaf structure of 18 cultivars of *C. japonica*, and showed that plants with a higher ratio of palisade tissues and a larger ratio of palisade tissues to spongy tissues in the leaf had better cold tolerance. Hainan Island is the largest tropical island in China and receives more light and heat because of its unique geographical location and climatic characteristics (Tang et al. 2008). We found that all of the tested genotypes from Hainan Island had fewer layers of palisade tissue cells than those from the Chinese mainland. This indicated that leaves of the tested genotypes exhibit constructive and physiological adaptability to their environment. Similar observa-
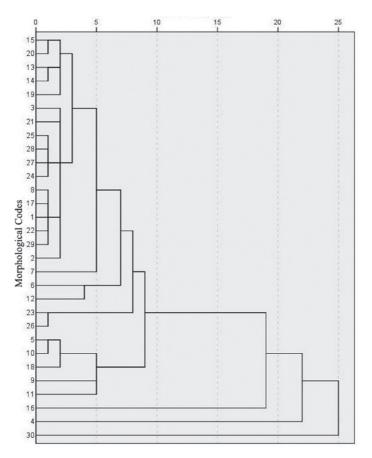
**Fig. 4. Cluster analysis of *Camellia* spp. based on leaf anatomy. Morphological codes correspond to characteristics listed in Table 1.**

tions were reported by Zou and Lou (1995).

For these *Camellia* genotypes, differences in leaf thickness were most directly related to differences in phylogeny. These results agreed with *Camellia* leaf anatomical features described by Deng et al. (1992). Since differences were observed in leaf thickness, palisade tissue layers, and other leaf characters, the *Camellia* genotype from the Chinese mainland (no. 30) was distinct from the 29 genotypes collected from the Hainan region (Fig. 4). This indicated that leaf characters of *Camellia* genotypes varied in different biogeographical regions, as previously reported by Lu et al. (2008b).

Anatomical differences were also ob-

served among the 29 genotypes originating on Hainan Island. In our study, 22 genotypes were collected from Chengmai County (nos. 1~22), 1 genotype was collected from Danzhou County (no. 23), 4 genotypes were from Qiongzhong County (nos. 24~27), and 2 genotypes were from Tunchang County (nos. 28 and 29) (Table 1). We observed that most of the genotypes from Chengmai County varied with their biogeographical distribution. As shown in Fig. 4 at a genetic distance of approximately 10, most *Camellia* genotypes from Chengmai County (nos. 1~3, 5~15, and 17~22) were assumed to be closely related, while nos. 4 and 16 were distinct. As shown in Fig. 4, at a genetic distance of approximately 22, no. 4

(Chengmai County) was in a separate group from the other genotypes and had a larger leaf thickness than the other genotypes from Hainan Island. At a data genetic distance of approximately 19, no. 16 was in a separate group than the other 27 genotypes and had larger lower epidermis cells. Xu et al. (2020) reported that Hainan oil-tea plants had close relative relationships with *C. oleifera* and represented a new species belonging to *Camellia* sect. *Oleifera* based on characters of morphology, pollen, and molecular phylogeny. We agree with the results of Xu et al. (2020) based on our leaf anatomical characteristics.

## CONCLUSIONS

In summary, although the 29 genotypes of oil tea from Hainan Island showed many similarities with *C. oleifera* native to the Chinese mainland, they varied in some characters especially in palisade tissue layers and leaf thickness. Our study provides leaf anatomical information for oil-tea taxonomic features and supports the view that these 29 genotypes belong to a new species *Camellia* sect. *Oleifera*.

## ACKNOWLEDGEMENTS

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