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Observations on leaf morphology of male and female *Actinidia chinensis* plants

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Abstract

Differences of leaf morphology between male and female plants of *Actinidia chinensis* were observed by means of microscopic and scanning electron microscope (SEM) observations. The experimental results showed that ratios of guard cell length to width were significantly different between male and female plants, which were greater than 3 in male plants and lower than 3 in female plants. Leaf shapes and petiole appearance were slightly different among different cultivars, however, the special parameter related to gender could not be found. Male seedlings and female seedlings germinated from seeds in the same fruit could be identified according to ratio of guard cell length to width. It is suggested that ratio of guard cell length to width may be used as a good marker to distinguish male plants from female plants in *A. chinensis*.

Key words: *Actinidia chinensis*, gender identification, leaf, morphology

Introduction

Kiwifruit plants are functionally dioecious plants and belong to *Actinidia* (Yang *et al*., 2010). There is gender diversity in *Actinidia* and the sex differentiation of kiwifruit plants is in primitive evolution, thus *Actinidia* can be used as the model plants for studies on sex origin, evolution, determination, and reproduction biology (Yang *et al*., 2009). Bugala in the 1950s, identified male and female *Populus tremula* by difference in leaf color (Li *et al*., 2006). Gong (1995) studied leaf shape, crown layer, bark and lenticel of the seedlings and mature plants of *Populus talassica* in summer and winter, and suggested the morphological parameters for gender identification. Lian *et al.* (2000) measured the leaves of *Hippophae rhamnoides* and found the female plants had usually larger ratio of leaf length to width than the male plants. To search for easy and rapid morphological markers for identifying gender of kiwifruit seedlings in their early developmental stage, comparisons of leaf morphology between male and female *Actinidia chinensis* were carried out in this study. The experimental results would be helpful in direct use of kiwifruit seedlings in farming production, selection of male or female plants in hybrid seedling population and cultivation of female vigorous F_1 plants.

Materials and methods

Plant materials: Four cultivars of *Actinidia chinensis* Planch var. chinensis, *viz.*, 'Heping Hongyang' ♂, 'Heping Hongyang' ♀, 'Fengxion No. 1' ♂ and 'Wuzhi No. 3' ♀, and 4 cultivars of *A. chinensis* Planch var. deliciosa, 'Bangzen No. 1' ♂, 'Heping No. 1' ♀, 'Zhaoxia No. 3' ♂ and 'Miliang No. 1' ♀, grown in the germplasm collection of Fruit Research Institute of Heping County (Guangdong, China), were used in this study. Six plants of each cultivar were randomly chosen for leaf samples. The mature green leaves were used for morphological observation.

Microscopic observation: Mature green leaves of vigorous shoots at different positions in each plant were picked up, photographed and determined. After observation on their leaf vein and leaf shape, the leaves were put in FAA solution (70% ethanol + 5% acetic acid + 2% formalin + 23% H_2O) for 24 h, and then transferred to 70% ethanol solution for long term storage. The fixed leaves were used to make freehand section, and the sections were scanned and photographed under Leica DM4000B microscope system.

SEM observation: The leaf samples were treated in FAA solution according to Yang (2010) for 24 h, and then transferred to 70% ethanol solution for long term storage. After dehydration in ethanol series (80%, 90%, 100%, for 10 min in each ethanol solution), the leaf samples were put in tertiary butanol 3 times (5 min each) and dried completely in a freeze dehydrator. The dry leaves were put on ion sputtering coator in which Pt was used as the ion source, and then observed under JSM-6360LV scanning electron microscope.

Results

Observations on leaf appearance of *A. chinensis*: One year old branch stems of *A. chinensis* Planch. var. chinensis were grey-green and the two year old branch stems were grey-brown, on which there was little or no hair. Yellow-brown lenticels protruded on the branch stems. Leaves were bifacial and the veins protruded on the lower surface (Fig. 1A-D). The blades were oval, round or round-fan shaped and the blade bases were heart-shaped and symmetrical. Terminal leaf margin of the blades was curved, obtuse-shaped or concave. The upper leaf surfaces were dark-green and smooth. The lower leaf surfaces were grey-green and hairy with pale-green hairy veins. The petioles were pale-violet or pale-green, without hair on petiole surface.

One year old branch stems of *A. chinensis* Planch var. deliciosa were green with grey-brown hairs and a few protruded lenticels on them, and the two year old branch stems were red-brown on

which there was no hair but a few pale oval-shaped lenticels on them. The leaves were bifacial leaves and the veins protruded on the lower surface (Fig. 1 E-H). The blades were round, oval or round-fan shaped, and the blade bases were shallow-heart shaped and symmetrical. The leaf margins showed small protruded spikes. The upper leaf surfaces were dark-green with a few yellow-brown villi. The lower leaf surfaces were pale-green with crowded yellow epidermal hairs. The leaf veins were yellow-green or pale-green, with short brown villi on them.

Size and shape of the leaves of *A. chinensis* showed some difference among different cultivars (Table 1). In cultivars of var. chinensis, the relative petiole length of the male plants was

Table 1. Morphological parameter of kiwifruit leaves (mm)

Cultivar	Petiole length	Petiole diameter	Leaf length	Leaf width
Bangzen No. 1 ♂	90.8±3.22	5.41±0.07	164.6±4.08	186.2±2.52
Zhaoxia No. 3 ♂	87.2±5.93	2.83±0.08	143.4±2.36	146.8±5.45
Fengxion No. 1 ♂	66.4±3.75	2.79±0.04	79.8±2.71	107.6±2.11
Heping Hongyang ♂	47.6±1.08	2.09±0.17	60.3±2.25	66.2±3.09
Heping No. 1 ♀	54.8±5.12	3.71±0.03	143.4±5.38	163.6±6.49
Miliang No. 1 ♀	70.2±6.84	3.51±0.12	125.2±4.43	137.6±4.37
Wuzhi No. 3 ♀	79.0±8.21	4.56±0.10	131.6±4.01	135.4±4.49
Heping Hongyang ♀	75.9±5.81	3.94±0.18	128.6±7.84	136.0±6.59

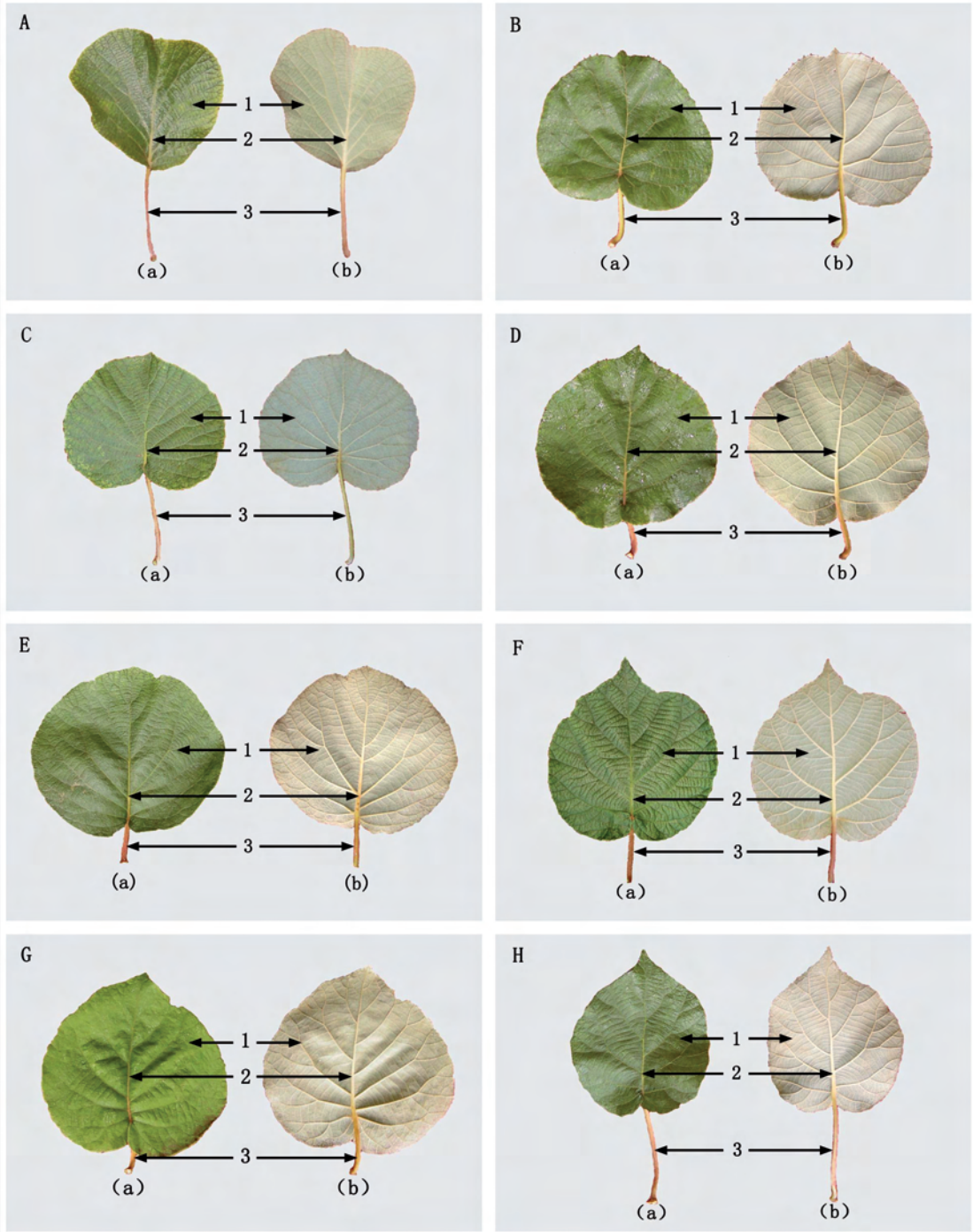

Fig. 1. Leaves of *A. chinensis*. A. Heping Hongyang ♂, B. Heping Hongyang ♀, C. Fengxion ♂ D. Wuzhi No. 3 ♀, E. Bangzen No. 1 ♂, F. Heping No. 1 ♀, G. Zhaoxia No. 3 ♂, H. Miliang No. 1 ♀, a. upper leaf surface, b. lower leaf surface, 1. side vein, 2. main vein, 3. petiole

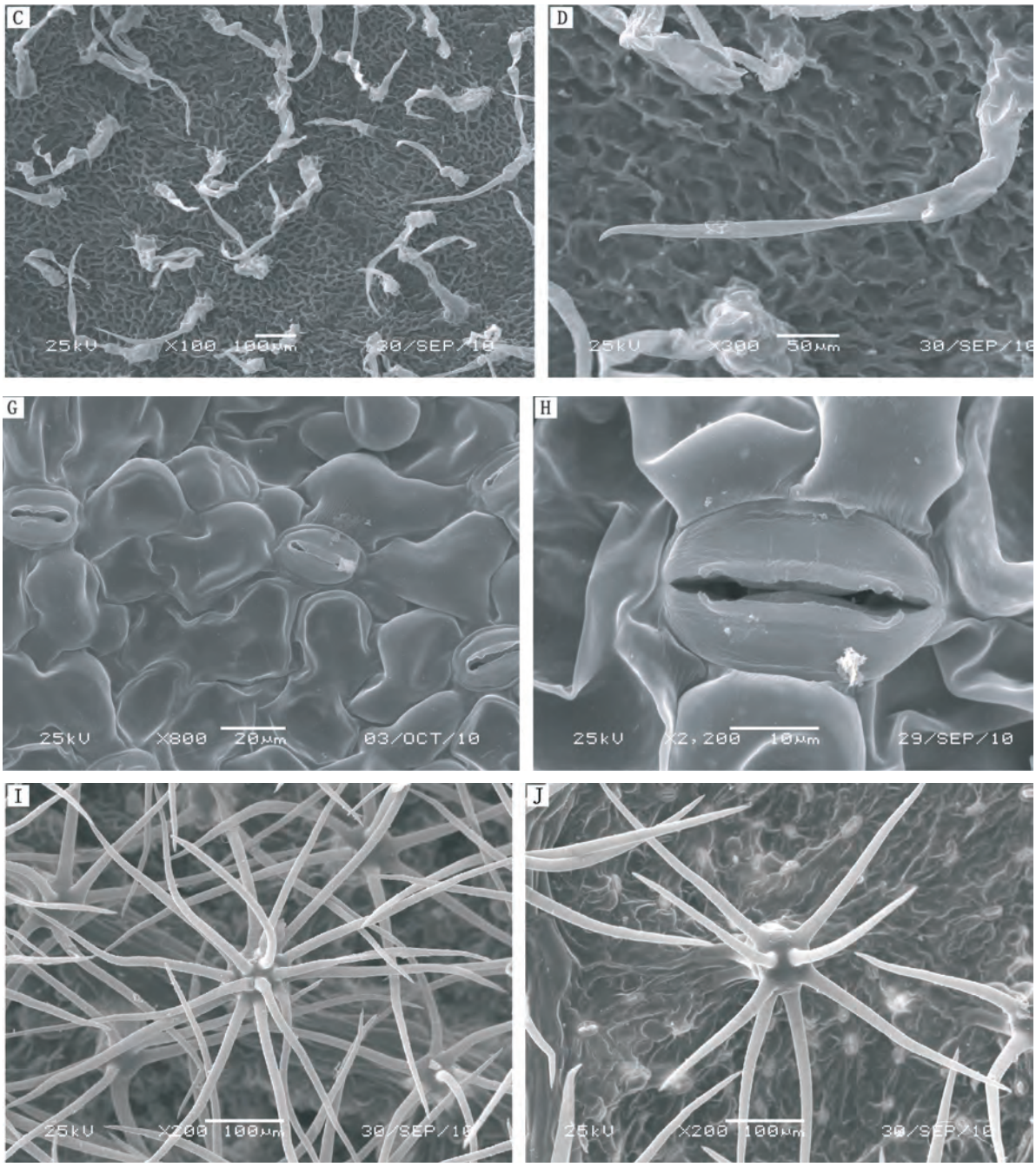

Fig. 2. SEM photographs of epidermis of *A. chinensis* leaves. C-D. upper epidermal hair, G-H. stoma in lower epidermis, I-J. lower epidermal hair

longer than the female plants, while in cultivars of var. deliciosa, the relative petiole length of the male plants was shorter than the female plants. In 8 cultivars studied, ratio of petiole length to petiole diameter of the male plants was more than the matched female cultivars (Bangzen No. 1♂> Heping No. 1♀, Zhaoxia No. 3♂> Miliang No. 1♀, Fengxion No. 1♂> Wuzhi No. 3♀, and Heping Hongyang♂> Heping Hongyang♀). The petioles of the male plants were relative long and thin. However, since there was variation and overlapping among cultivars or combinations, parameters of leaf shape and petiole appearance could not be used as maker to distinguish male plants from female plants.

Microscopic observation on *A. chinensis*: Sections of leaf blades from 8 cultivars of *A. chinensis* under microscope showed upper epidermis, palisade parenchyma, spongy parenchyma and lower epidermis. The upper epidermis was of one layer of cells with thickness of 14-22 μm. The palisade parenchyma was one to two layers of cells, arranged closely. The spongy parenchyma arranged irregularly. The lower epidermis consisted of 1-5 layers of loosely arranged cells. Leaf veins interspersed among spongy parenchyma and protruded outside. Anatomically, there was little difference between var. chinensis and var. deliciosa, and also little difference between the male plants and the female plants.

SEM observations on *A. chinensis*: SEM observations on *A. chinensis* showed that there were few and scattered trichomes, seldom branched on the upper leaf surfaces (Fig. 2C-D). The lower leaf surface of var. chinensis spread thick trichomes and villi. Branches of a trichome were 3-19 (Fig. 2I-L). The SEM observations showed little difference among different cultivars and also little difference between the male plants and the female plants.

There was no stoma at the upper leaf surface in the 8 cultivars of *A. chinensis*. Each of the stomas in the lower leaf surface was enclosed by two kidney-shaped guard cells. Measurement of the guard cells showed that appearance of the guard cells was closely related to sexual distinction. The ratios of guard cell length to their width was greater than 3 in all the 4 male cultivars, and the ratio was less than 3 in all the 4 female cultivars (Table 2). Similar phenomenon was also present in the seedling plants germinated from the seeds of "Heping No. 1" × "Bangzen No. 1" and "Wuzhi No. 3" × "Fengxion No. 1" (Table 3). Thus, the ratio of guard cell length to width could be used as a reliable marker to distinguish male plants and seedlings from female plants and seedlings.

Table 2. Length and width of the guard cells of *A. chinensis*

Cultivar	Length (μm)	Width (μm)	Length / width
Bangzen No. 1 ♂	31.6	9.1	3.5
Zhaoxia No. 3 ♂	28.9	6.2	4.7
Fengxion ♂	27.8	9.1	3.1
Heping Hongyang ♂	18.4	5.6	3.3
Heping No. 1 ♀	22.7	8.9	2.6
Miliang No. 1 ♀	19.1	7.8	2.5
Wuzhi No. 3 ♀	31.1	10.9	2.9
Heping Hongyang ♀	20.1	6.9	2.9

Table 3. Length and width of the guard cells of *A. chinensis* seedlings

Cross combination	Length (μm)	Width (μm)	Length / width
Heping No. 1 × Bangzen No. 1 (♂)	26.7±3.4	7.5±1.1	3.6±0.3
Heping No. 1 × Bangzen No. 1 (♀)	24.1±2.4	8.9±0.7	2.7±0.2
Wuzhi No. 3 × Fengxion No. 1 (♂)	26.1±1.7	7.8±0.2	3.4±0.2
Wuzhi No. 3 × Fengxion No. 1 (♀)	24.9±0.7	8.7±0.9	2.9±0.1

Discussion

It has long been thought that *A. chinensis* and *A. deliciosa* belong to different species (Li *et al.*, 2007). However, many studies suggested that the cultivars of these two taxa overlapped in their morphological characters and molecular markers (Chen *et al*., 2008; Chen *et al.*, 2005; Huang *et al.*, 2000; Huang *et al*., 1999; Cui.1993). Now it is believed that these two taxa are different varieties of the same species, *A. chinensis* Planch var. *chinensis* and *A. chinensis* Planch var. *deliciosa* (Huang, 2009). Some differences were present in the cultivars between var. chinensis and var. deliciosa, and also present in the plants between male and female (Yang *et al.*, 2009; Liu *et al*., 2006; Olah, 1997). Based on this study, the relative petiole length of male var. chinensis was longer than the female one, while that of male var. deliciosa was shorter than the female one. In both varieties, the ratio of petiole length to petiole diameter of the female plants was less than the matched male plant. The male petioles were relatively longer and thinner. Since leaf appearance of kiwifruit plants is quantitative character easy to be affected by nutritional, environmental and physiological factors, it is difficult to distinguish male plants from female ones according to the leaf appearance.

The study revealed that the ratio of guard cell length to width significantly discriminated male and female plants. Thus the ratio of guard cell length to width could be used to effectively distinguish male plants from female plants both in seedlings and mature plants.

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References

Chen, X.L., H. Liang and Z.W. Xie, 2008. Cluster analysis of fruit and leaf characters from 11 species of *Actinidia*. *Journal Anhui Agricultural Sciences*, 36(35): 15408-15410 (in Chinese).

Chen, X.L., Z.W. Xie and H. Liang, 2005. Genetic diversity of *Actinidia* in Heping County of Guangdong Province. *Advance in Actinidia Research*, Beijing: China Science Press, 148-157 (in Chinese).

Cui, Z.X. 1993. *China Kiwifruit*. Jinan: Shangdong Science and Technology Press, p. 6-66. (in Chinese)

Gong, W.L. 1995. Direct identification of male and female plants of *Populus talassica*. *Tianjin Agriculture and Forestry Science and Technology*, 2: 40-41. (in Chinese)

Huang, H.W. 2009. History of 100 Years of domestication and improvement of kiwifruit and gene discovery from genetic introgressed populations in the wild. *Chinese Bulletin Botany*, 44 (2): 127142.

Huang, H.W., J. Gong and S.M. Wang, 2000. Genetic diversity of *Actinidia*. *Biodiversity Science*, 8(1): 49-54. (in Chinese)

Huang, H.W., J. Li, P. Lang and S.M. Wang, 1999. Systematic relationships in *Actinidia* as revealed by cluster analysis of digitized morphological descriptors. *Acta Hort.*, 498: 71-78.

Li, J.Q., X.W. Li and D.D. Soejarto, 2007. Actinidiaceae. In: *Flora of China*. Vol.12. Z.Y. Wu, P.H. Raven, D.Y. Hong (Eds.), Beijing: Science Press & Saint Louis, Missouri: Missouri Botanical Gardens. pp. 334-360.

Li, R.L., L.D. Lu, W.J. Gao, S.F. Li and Q. Wang, 2006. Advances in sex identification of dioecious plants. *Guihaia*, 26(4): 387-391. (in Chinese)

Lian, Y.S., S.G. Lu and S.K. Xie, 2000. *Biology and Chemistry of Hippophae*. Langzhou: Gansu Science and Technology Press, p. 176-184. (in Chinese)

Liu Y.L., Z. Li, P.F. Zhang, Z.W. Jiang and H.W. Wang, 2006. Spatial genetic structure in natural populations of two closely related *Actinidia* species (Actinidiaceae) as revealed by SSR analysis. *Biodiversity Science*, 14(5): 421-434. (in Chinese)

Olah, R., E. Masarovicova and J. Samj, 1997. Anatomical and morphological parameters of leaves and leaf petioles of *Actinidia deliciosa*. *Biologia Plantarum*, 39(2): 271-280.

Yang, M.X. 2010. *Cytomorphological Observation on Sex Differentiation of Actinidia Chinensis*. MS degree thesis, Zhongkai University of Agriculture and Engineering, (in Chinese).

Yang M.X., H. Liang and S.D. He, 2009. Research progress in sex differentiation and identification of Actinidia. *Journal Zhongkai University Agriculture Engineering*, 22(1): 57-60. (in Chinese)

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