African Journal of Biotechnology Vol. 10(62), pp. 13470-13475, 12 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2248
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Dynamic changes of stomatal characteristics during the flower, fruit and leaf developments of *Zephyranthes candida* (Lindl.) Herb

Zhaoyu Jiang#, Juntong Yu#, Sanmei Ma and Yongfei Wang*

College of Life Science and Technology, Jinan University, Guangzhou 510632, P. R. China.

Accepted 20 May, 2011

The presence of stomata in flower and fruit is common but there is limited information about stomata distribution during their development. The aim of this study was to verify the stomatal characteristics in the petal surfaces, exocarps and leaf epidermis during their developments. The median region of exocarps, adaxial and abaxial epidermis of the petals and leaf midribs during the development of *Zephyranthes candida* was studied under light microscopes. The results showed that there were stomata on the median region of exocarps, adaxial and abaxial epidermis of the petals and leaf midribs. The petal and fruit epidermal cells were polygonal in shape, while leaf epidermal cells were strip. The leaf stomatal index and stomatal density were the largest in the surfaces investigated. In different parts, the stomatal index, stomatal density, length and width of guard cell showed different correlations during different stages of development.

Key words: Stomatal density, stomatal index, guard cell, development, correlation.

INTRODUCTION

Stomata are pores in the plant epidermis, surrounded by two guard cells, which control the aperture opening and closing. Stomata are specialized epidermal structures that regulate CO_2, O_2 and water vapor exchange between plants and their environment (Evert, 2006). Although, there are many studies on leaf stomata, some have reported on non-photosynthetic organs, such as petals (Azad et al., 2007), fruits (Peschel et al., 2003; Zieliński et al., 2010), seeds (Paiva et al., 2006), nectaries (Davies et al., 2005; Horner et al., 2003) and primary roots (Christodoulakis et al., 2002). Stomata are closely related with environmental changes, global climate changes (Hetherington and Woodward, 2003; Berry et al., 2010) and plant immunity (Melotto et al., 2006), apart from controlling of gas exchange. Since stomata play such important roles, stomatal distribution and function in different parts of plant have aroused wide concern. *Zephyranthes candida* (Lindl.) Herb (Fairy Lily; Amaryllidaceae) is a perennial herb. The leaf is glossy deep green, linear and about 3 mm wide. The white flower is erect in perianth, the scape is slender and hollow and the flower is single born on top of the scape. The bottom of the spathe-shaped involucrum is tubular. The capsule is nearly spherical and the seed is black and flat. There are lots of studies on the pharmacology of *Z. candida* (Mutsuga et al., 2001; Wu et al., 2009) but few are on the stomata distribution of different organs during development. In this study, the stomata distribution of petals, fruits and leaves during development were investigated to provide some new morphological information on *Z. candida*.

*Corresponding author. E-mail: wyfmsm@163.com. Tel: 86-020-38897606.

#Both authors contributed equally to this work.

MATERIALS AND METHODS

Z. candida (Lindl.) Herb used in this study was grown at the campus of Jinan University, China. The different developmental stages of flower, fruit and leaf were collected. The petals, in different stages of Flower development, were categorized into the following eight developmental stages by the petal length: 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 cm. The fruit were categorized into the following 6 developmental stages by the fruit diameter: 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 cm. The leaves were categorized into the following 8 developmental stages by the leaf length: 10, 15, 20, 25, 30, 35, 40 and 45 cm.

Measurement of stomatal characteristics

Fresh flowers, fruits and leaves were selected for observation. Samples were taken from the median region of exocarps, adaxial and abaxial epidermis of the petals and leaf midribs. The plant materials were washed with deionized water. The temporary slides were obtained by the way of ripping off or the nail polish blot from the materials. The number of stomata (NS), number of epidermal cells (NE), the guard cell length (GCL) and guard cell width (GCW) were determined using a light microscope (NIKON YS100, Japan) at 400× magnification. Fifty (50) fields were randomly counted from 10 plants for each part. Each experiment was repeated 3 times. The stomatal density (SD) and stomatal index (SI) were estimated. SD referred to the number of stomata per squared millimeter. SI was calculated using the formula NS / (NE + NS) × 100% (Cutter, 1978).

Data analysis

The average and standard error values of SD, SI, GCL and GCW were calculated and compared among the different organs using ANOVA. Student-Newman-Keuls' test was used to determine differences between treatments. Correlations among SD, SI, GCL and GCW were tested using Pearson correlation coefficient.

RESULTS

Epidermal characteristics of different parts of *Z. candida*

The epidermal cell characteristics of the flower, fruit and leaf of *Z. candida* are shown in Figure 1. There were stomata on the median region of exocarps, adaxial and abaxial epidermis of the petals and leaf midribs. The patterns of anticlinal walls of epidermal were straight. The petal epidermal cells were polygonal in shape. The petal guard cells were kidney-shaped and some were deformed for extrusion from the adjacent epidermal cells. The exocarp epidermal cells were also polygonal in shape. The exocarp guard cells were kidney-shaped. The leaf epidermal cells were strip-shaped and arranged in neat rows. The leaf epidermal guard cells were kidney-shaped and their growth axes were parallel with the epidermal cells.

Stomatal characteristics during the petals development

The stomatal characteristics of the adaxial and abaxial epidermis during the petals growth from 1.5 to 5 cm are summarized in Table 1. During the flower development, stomatal characteristics changed. The stomatal index and stomatal density on the lower petal surface were larger than on the upper petal surface at the same development stage.

On the adaxial epidermis, the SI increased at first, and then decreased. When petal length was 3.0 cm, the SI reached its maximum (SI is 0.92 ± 0.11%). The SD decreased remarkably with the petal growth. The size of stomata was (25.60 -34.64) μm x (5.99 - 9.40) μm. Similarly, on the abaxial epidermis, the SI began to increase and then decreased with the petal growth, and had a maximum value at a critical petal length of 4.0 cm. The SD also decreased remarkably. The size of stomata was (22.18 - 33.05) μm × (4.86 - 9.25) μm. When petal length was 4.5 cm, the GCW on both surfaces reached its maximum, respectively.

Stomatal characteristics during the fruits development

The stomatal characteristics of the median region of the excarps during the fruits growth from 0.4 to 1.4 cm in diameter are shown in Table 2. The stomatal characteristics changed with the fruits development. The SI, SD and GCW decreased with the fruit development. When fruit diameter was 0.4 cm, the SI, SD and GCW reached their maximum, respectively. The size of the stomata on the fruit was (30.13 - 33.20) μm × (10.65 - 13.59) μm.

Stomatal characteristics during the leaves development

The stomatal characteristics on the adaxial and abaxial epidermis during the leaves growth from 10 to 45 cm in length are summarized in Table 3. The stomatal characteristics changed with the leaf development. The SI and SD on both surfaces varied in waves. On the adaxial epidermis, when leaf length was 25 cm, the SI and SD were at their minimum, respectively. The size of stomata was (40.12 - 48.24) μm × (11.18 - 15.35) μm. On the abaxial epidermis, when leaf length was 40 cm, the SI and SD had a minimum respectively. The size of the stomata was (42.00 - 53.01) μm × (10.83 - 15.40) μm.

Relationships among stomatal index, stomatal density, length and width of guard cell

As shown in Table 4, during petals extension from 1.5 to 5.0 cm, there was a very significant negative correlation between the SD and GCL ($P < 0.01$). A significant negative correlation was recorded between the SD and GCW ($P < 0.05$). There was a very significant positive correlation between the GCL and GCW ($P < 0.01$). According to the information in Table 5, a conclusion could be drawn that there was an extremely significant

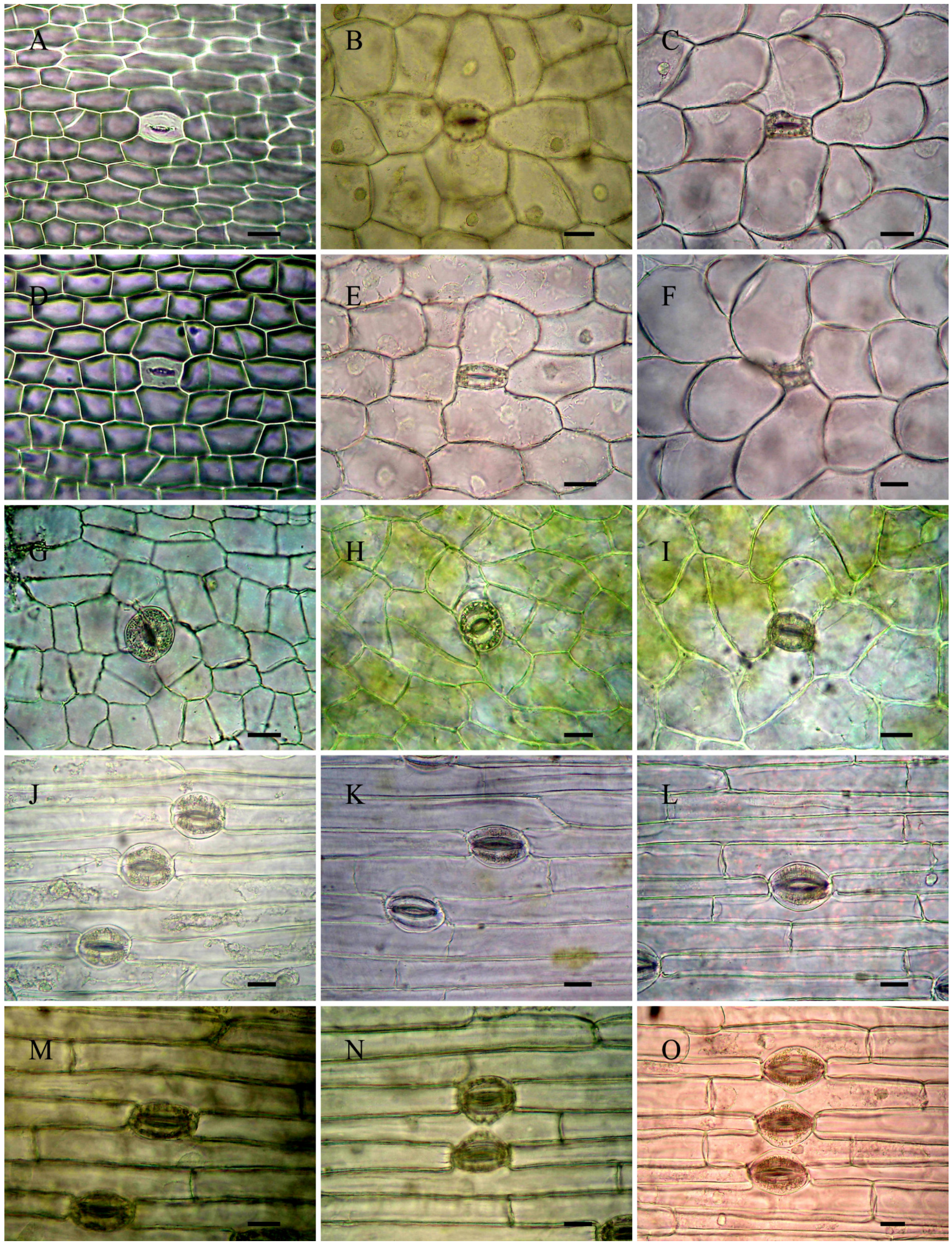

Figure 1. Epidermal characteristics of the flower, fruit and leaf of *Z. candida.* A to C, the upper surface of the different length of petals: A, 1.5 cm; B, 3.5 cm; C, 5.0 cm. D to F, the lower surface of the different length of petals: D, 1.5 cm; E, 3.5 cm; F, 5.0 cm. G to I, exocarps of the different diameter: G, 0.4 cm; H, 0.8 cm; I, 1.4 cm. J to L, the adaxial epidermis of the different length of leaves: J, 10 cm; K, 25 cm; L, 45 cm. M to O, the abaxial epidermis of the different length of leaves: M, 10 cm; N, 35 cm; O, 45 cm. Scale bar = 20 μm.

positive correlation between the SI and SD during the fruit diameter change from 0.4 to 1.4 cm ($P < 0.01$).

As shown in Table 6, during the leaf extension from 10 to 45 cm, there was an extremely significant positive correlation between the SI and SD ($P < 0.01$). A significant negative correlation was observed between the SI and GCL ($P < 0.05$).

DISCUSSION

Few researches have been done on the stomata of plant reproductive organs. There are stomata in the flower and fruit of *Z. candida*. The characteristics of stomata on the epidermis changed correspondingly with the development of flower and fruit. The SI on the two surfaces of the petal

Table 1. Stomatal characteristics of the different petals length.

Petal length (cm)	SI (%)	SD (mm^{-2})	GCL (μm)	GCW (μm)
Upper surface				
1.5	0.54^{b}±0.04	16.48^{a}±1.12	25.76^{c}±0.45	7.48^{bc}±0.31
2.0	0.56^{b}±0.05	11.57^{b}±1.04	25.88^{c}±0.65	5.99^{d}±0.25
2.5	0.63^{ab}±0.03	9.81^{b}±0.45	25.60^{c}±0.42	8.63^{ab}±0.26
3.0	0.92^{a}±0.11	7.42^{c}±0.86	25.60^{c}±0.57	7.51^{bc}±0.35
3.5	0.52^{b}±0.08	2.77^{d}±0.45	34.64^{a}±0.75	8.38^{ab}±0.37
4.0	0.73^{ab}±0.11	3.77^{d}±0.57	31.56^{b}±0.61	6.40^{cd}±0.45
4.5	0.74^{ab}±0.12	3.90^{d}±0.62	36.00^{a}±0.44	9.40^{a}±0.37
5.0	0.52^{b}±0.10	2.26^{d}±0.43	30.56^{b}±0.74	7.81^{b}±0.24
Lower surface				
1.5	0.90^{c}±0.06	20.88^{a}±1.24	22.53^{e}±0.44	4.86^{d}±0.37
2.0	1.08^{bc}±0.09	16.10^{b}±1.30	25.11^{d}±0.47	6.00^{c}±0.26
2.5	0.81^{c}±0.08	11.32^{c}±1.08	22.18^{e}±0.47	4.97^{d}±0.21
3.0	1.34^{ab}±0.12	8.55^{d}±0.76	27.15^{c}±0.41	5.54^{cd}±0.19
3.5	1.55^{a}±0.15	7.37^{de}±0.69	31.28^{a}±0.38	7.25^{b}±0.30
4.0	1.58^{a}±0.16	6.29^{de}±0.62	32.41^{a}±0.87	8.08^{b}±0.18
4.5	1.38^{ab}±0.12	5.66^{de}±0.52	33.05^{a}±0.42	9.25^{a}±0.45
5.0	0.93^{c}±0.12	3.52^{e}±0.45	29.43^{b}±0.82	8.20^{b}±0.36

For each column, values which have the same letter are not significantly different at the 0.05 level as determined by Student-Newman-Keuls' test. SI, Stomatal index; SD, stomatal density; GCL, guard cell length; GCW, guard cell width.

Table 2. Stomatal characteristics of the different fruits diameter.

Fruit diameter (cm)	SI (%)	SD (mm^{-2})	GCL (μm)	GCW (μm)
0.4	1.29^{a}±0.10	18.87^{a}±1.63	30.13^{b}±0.72	13.59^{a}±0.31
0.6	0.69^{b}±0.07	8.30^{b}±0.91	33.14^{a}±0.72	13.18^{a}±0.40
0.8	0.61^{b}±0.08	4.78^{c}±0.58	32.33^{ab}±0.44	13.22^{a}±0.35
1.0	0.64^{b}±0.07	4.53^{c}±0.54	31.47^{ab}±0.39	10.92^{b}±0.41
1.2	0.68^{b}±0.09	4.28^{c}±0.55	33.20^{a}±0.45	10.73^{b}±0.47
1.4	0.49^{b}±0.08	3.27^{c}±0.52	32.35^{ab}±0.45	10.65^{b}±0.37

For each column, values which have the same letter are not significantly different at the 0.05 level as determined by Student-Newman-Keuls' test.

increased at first and then decreased, while the SD was reduced. The SI and SD on the exocarp generally decrease with the development of the fruit. The SD on the exocarp decreased sharply at first and then slowed down gradually. Yamada et al. (2009), researched on the development of the rose petal. They found that the epidermal cell division was dominant at the early stage, while the epidermal cell expansion was dominant at the later period. In this study, the epidermal and guard cell division are dominant in the initial period of development and then these two kinds of cells expansion are dominant, causing SD reduction.

Leaf is a plant vegetative organ. The stomata on the leaf epidermis have been studied extensively. Some have stomata on the abaxial and adaxial epidermis. In the terrestrial plant, the SD is larger on the abaxial epidermis than on the adaxial (Wu et al., 2005). But in this study, the SI and SD on the adaxial leaf epidermis are larger than on the abaxial, and this is consistent with *Trifolium repens* (Zoric et al., 2009). This may be due to the fact that *Z. candida* grows in the moist soil. The stomatal density and index on the adaxial epidermis are large to speed up the evaporation, which could be supply sufficient traction for the transport of nutrients and could more effectively reduce the surface temperature of plants at a high temperature.

The SD refers to the number of stomata per squared millimeter. Overall, the SI and SD on the leaf are much larger than those on the petal and fruit. The stomata regulate CO_2 and O_2 exchange, which will affect plant photosynthesis and respiration. Leaf, the plant vegetative organ needs much stomata in order to carry out

Table 3. Stomatal characteristics of the different leaves length.

Leaf length (cm)	SI (%)	SD (mm^{-2})	GCL (µm)	GCW (µm)
Adaxial epidermis				
10	20.04^{a}±0.35	75.22^{a}±1.90	40.12^{c}±0.76	12.76^{bc}±0.25
15	16.90^{b}±0.40	61.38^{b}±1.89	42.52^{b}±0.28	12.16^{bcd}±0.26
20	15.33^{c}±0.43	50.06^{cd}±1.56	43.21^{b}±0.32	12.15^{bcd}±0.27
25	10.50^{g}±0.27	36.48^{f}±0.95	43.05^{b}±0.49	11.73^{cd}±0.47
30	14.27^{d}±0.40	54.84^{c}±1.88	46.84^{a}±0.30	12.95^{b}±0.20
35	13.55^{de}±0.32	44.28^{e}±1.28	48.24^{a}±0.58	13.30^{b}±0.24
40	11.48^{f}±0.21	50.31^{cd}±1.02	43.11^{b}±0.53	15.35^{a}±0.13
45	12.98^{e}±0.30	46.04^{de}±1.04	48.21^{a}±0.37	11.18^{d}±0.41
Abaxial epidermis				
10	16.12^{a}±0.35	54.34^{a}±1.37	42.00^{e}±0.31	10.83^{e}±0.28
15	13.77^{b}±0.42	40.00^{b}±1.29	42.38^{e}±0.34	11.74^{cd}±0.29
20	12.30^{cd}±0.47	27.42^{de}±1.16	42.43^{e}±0.37	12.61^{c}±0.37
25	10.72^{e}±0.30	30.19^{d}±1.05	42.57^{e}±0.53	11.01^{de}±0.21
30	13.28^{bc}±0.41	41.13^{b}±1.58	45.10^{c}±0.34	12.40^{c}±0.26
35	10.54^{e}±0.26	34.47^{c}±0.88	46.31^{b}±0.38	12.11^{c}±0.27
40	8.22^{f}±0.25	25.66^{e}±0.76	53.01^{a}±0.38	15.40^{a}±0.18
45	11.57^{de}±0.36	34.72^{c}±1.25	52.80^{a}±0.28	13.55^{b}±0.28

For each column, values which have the same letter are not significantly different at the 0.05 level as determined by Student-Newman-Keuls' test.

Table 4. Correlation matrix of stomatal characteristics in the petals development.

Parameter	SI	SD	GCL	GCW
SI	1	-0.051	0.161	0.20
SD	-	1	-0.791**	-0.615*
GCL	-	-	1	0.727**
GCW	-	-	-	1

* Correlations are significant at $P < 0.05$; ** correlations are significant at $P < 0.01$.

Table 5. Correlation matrix of stomatal characteristics in the fruits development.

Parameter	SI	SD	GCL	GCW
SI	1	0.973**	-0.755	0.592
SD	-	1	-0.722	0.693
GCL	-	-	1	-0.328
GCW	-	-	-	1

* Correlations are significant at $P < 0.05$; ** correlations are significant at $P < 0.01$.

photosynthesis and respiration to provide adequate organic matter and energy for the plant. Flower and fruit are the plant reproductive organs, which makes the plant to reproduce. The function of the flower and fruit stomata is still uncertain. Hew et al. (1980), found that the stomata in the flowers of tropical orchids were incapable of photosynthesis and transpiration. Azad et al. (2007) reported that the stomata on the epidermis of the tulip petals could open and close according to the temperature, to control the water balance. Whether the stomata on the petal epidermis of *Z. candida* can carry out photosynthesis and transpiration needs to be studied

Table 6. Correlation matrix of stomatal characteristics in the leaves development.

Parateter	SI	SD	GCL	GCW
SI	1	0.898**	-0.560*	-0.324
SD	-	1	-0.463	-0.106
GCL	-	-	1	0.477
GCW	-	-	-	1

* Correlations are significant at P < 0.05; ** correlations are significant at P < 0.01.

further. Peschel et al. (2003) reported that the stomata on the exocarp of sweet cherry were functional before maturity but non-functional at maturity. Whether the stomata on the petal epidermis of *Z. candida* are functional also needs to be studied further.

During the development of flower, fruit and leaf, different relevance was shown among the stomatal characteristics. In the process of flower development, the SD and GCL showed a very significant negative correlation ($P < 0.01$), and the length and width of guard cells showed a very significant positive correlation ($P < 0.01$). At the early flower development, the SD was relatively large, while the guard cell was relatively small. But as the flower grew, the SD decreased, while the guard cell became larger. During the fruit and leaf development, the SD and SI showed a highly significant positive correlation ($P < 0.01$), and the SD and SI changed synchronously.

Acknowledgements

This research was supported by the grant from the Key Research Project of Science and Technology of Guangdong Province of China (No. 2011B020303006).

REFERENCES

Azad AK, Sawa Y, Ishikawa T, Shibata H (2007). Temperature-dependent stomatal movement in tulip petals controls water transpiration during flower opening and closing. Ann. Appl. Biol., 150(1): 81-87.

Berry JA, Beerling DJ, Franks PJ (2010). Stomata: key players in the earth system, past and present. Curr. Opin. Plant Biol., 13(3): 233-240.

Christodoulakis NS, Menti J, Galatis B (2002). Structure and development of stomata on the primary root of *Ceratonia siliqua* L. Ann. Bot., 89(1): 23-29.

Cutter EG (1978). Plant anatomy. London: Edward Arnold, 313.

Davies KL, Stpiczynnska M, Gregg A (2005). Nectar-secreting floral stomata in *Maxillaria anceps* Ames & C. Schweinf. (Orchidaceae). Ann. Bot., 96(2): 217-227.

Evert RF (2006). Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development, 3rd edn.Hoboken, New Jersey: John willey & Sons Inc. pp. 214-221

Hetherington AM, Woodward FI (2003). The role of stomata in sensing and driving environmental change. Nature, 424(6951): 901-908.

Hew CS, Lee GL, Wong SC (1980). Occurrence of nonfunctional stomata in the flowers of tropical orchids. Ann. Bot., 46(2): 195-201.

Horner HT, Healy RA, Cervantes-Martinez T, Palmert RG (2003). Floral nectary fine structure and development in *Glycine max* L. (Fabaceae). Int. J. Plant Sci., 164(5): 675-690.

Melotto M, Underwood W, Koczan J, Nomura K, He SY (2006). Plant stomata function in innate immunity against bacterial invasion. Cell, 126(5): 969-980.

Mutsuga M, Kojima K, Nose M, Inoue M, Ogihara Y (2001). Cytotoxic activities of alkaloids from *Zephyranthes carinata*. Nature Med., 55(4): 201-204.

Paiva EAS, Lemos-Filho JP, Oliveira DMT (2006). Imbibition of *Swietenia macrophylla* (Meliaceae) seeds: the role of stomata. Ann. Bot., 98(1): 213-217.

Peschel S, Beyer M, Knoche M (2003). Surface characteristics of sweet cherry fruit: stomata-number, distribution, functionality and surface wetting. Sci. Hort., 97(3-4): 265-278.

Wu D, Wang H, Lu JM, Zhu LD (2005). Comparative morphology of leaf epidermis in *Parnassia* (Parnassiaceae) from China. Acta Phytotaxon. Sin., 43(3): 210-224.

Wu ZP, Chen Y, Xia B, Wang M, Dong YF, Feng X (2009). Two novel ceramides with a phytosphingolipid and a tertiary amide structure from *Zephyranthes candida*. Lipids, 44(1): 63-70.

Yamada K, Norikoshi R, Suzuki K, Nishijima T, Imanishi H, Ichimura K (2009). Cell division and expansion growth during rose petal development. J. Jpn. Soc. Hort. Sci., 78(3): 356-362.

Zieliński J, Tomaszewski D, Guzicka M, Maciejewska-Rutkowska I (2010). Stomata on the pericarp of species of the genus *Rosa* L. (Rosaceae). Plant Syst. Evol., 284(1-2): 49-55.

Zoric L, Merkulov L, Lukovic J, Boza P, Polic D (2009). Leaf epidermal characteristics of *Trifolium* L. species from Serbia and Montenegro. Flora, 204(3): 198-209.