TITLE:

Ontogenesis of D-type stomata and cork-warts on the leaf epidermis of *Camellia japonica* (Theaceae) and functional assessment

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ABSTRACT

Cork-warts are particular structures forming round areas by radially arranged concentric rows of suberized cells, and have been found in many flowering plant genera. The roles of large D-type stomata and cork-warts in plant blades remained unclear for many years. To distinguish the large D-type stomata from cork-warts and identify their possible functions, both the lower epidermis and cross-sections of leaves were studied in *Camellia japonica* L. Simultaneously, Fourier Transform Infrared Spectroscopy (FTIR) technology was used to assess whether the cork-warts were really the structures accumulating suberin. Furthermore, the gas exchange and water evaporation functions of D-type stomata and cork-warts were measured. The results showed that D-type stomata and cork-warts had the same origin but they developed into two separate functional structures at different developmental stages. FTIR spectroscopy revealed that the cork-warts accumulated more suberin with phenolic domains, and more cell wall polysaccharide than matured typical stomata and cork-wart protomeristem. It is proposed that the D-type stomata and cork-warts are responsible for air exchange and water evaporation and their presence might correlate with the adaptation of *Camellia* species to the more humid understory environment.

KEYWORDS: air exchange, cork-wart, genus *Camellia*, large D-type Stomata, water

evaporation.

1. Introduction

Cork-warts were described as particular structures forming round areas by radially arranged concentric rows of suberized cells, normally found on upper and lower epidermis of leaves (Farooqui, 1982; Joffily and Cardoso Vieira, 2010). They often can be observed as black or brown spots on matured foliar lamina, and were initially described at the end of the 19th century (Poulsen, 1875; Matteucci, 1897). This type of structure has been reported on the leaves of a handful of angiosperm taxa, such as *Ilex* L. (Aquifoliaceae) (Korn and Fredrick, 1973), *Eucalyptus* L'Hér. (Farooqui, 1982), *Rhizophora* L. (Evans et al., 2005), several genera in Celastraceae (e.g. *Euonymus* L., *Celastrus* L., *Goniodiscus* Kuhl.) (Metcalf and Chalk, 1950; Joffily and Cardoso Vieira, 2010) and *Camellia* L. (Sealy, 1958; Chang, 1996; Gao et al., 2005).

Although cork-warts have first been reported more than a hundred years ago, their origin and function remained unclear. Farooqui (1982) attributed their origin to stomata development, while others consider them as random mechanical injuries or insect punctures (Haberlandt, 1914; Stace, 1965). The cork-warts in *Ilex crenata* Thunb. var. *convexa* Rehder have been interpreted as wound responses to the deterioration of large stomata, which are defined as D-type stomata (Korn and Fredrick, 1973). These large stomata are few in number, arise early during development, and are relatively short-lived compared to typical leaf stomata. However, this wound response hypothesis can hardly explain the phenomenon that most cork-warts are present in healthy leaves. Besides, there is no direct evidence that cork-warts originated from D-stomata, although they are located in the same position. Dickison (2000) correlated these structures with a defense system developed by plants to heal and repair mechanical injuries. Recently, Evans et al. (2005; 2010) reported that cork-warts contributed to the internal airflow from leaves to substrate roots in the mangrove species *Rhizophora*. Although the occurrence of cork-warts is interesting and peculiar, there is limited information on the functions compared to typical leaf stomata, and their developmental relationship between D-type stomata is still unclear. Thus, more targeted research is needed on their ontogeny and possible physiological functions.

Cork-warts in *Camellia* (Theaceae) plants are present on the abaxial leaf epidermis. The presence has been frequently reported in understory *Camellia* species (Keng, 1962; Parks and Griffiths, 1963; Chang, 1996, 1998; Ming, 2000; Shen et al., 2008; Jiang et al., 2010), but the descriptions varied due to the lack of anatomical evidence. More importantly, these leaf anatomic characters are stable in certain species and sections in *Camellia*, and it was considered as an important systematic

and diagnostic value in this genus (Chang, 1998; Ming, 2000; Jiang et al., 2010). In the present study, we first used anatomical methods to investigate the ontogeny of cork-warts and D-type stomata in *Camellia japonica* L. leaves, then explored the functions of these structures with respect to gas exchange and water evaporation through comparison with the non-cork-wart bearing species *C. reticulata* Lindl. Finally, we compared their cell wall composition among cork-wart protomeristem (CWP), mature typical stomata and cork-warts using FTIR micro-spectroscopy.

2. Materials and methods

2.1 Plant materials and epidermal scrapings

Camellia japonica and *C. reticulata* leaf samples were obtained from the Jinhua International *Camellia* Species Garden (Jinhua City, China, 29°7' N and 119°35' E). *Camellia japonica* samples were collected at six developmental stages: leaf bud stage, before leaf expansion, partly expanded leaf, fully expanded leaf, D-type started to decay, and cork-warts well developed stage. The development stages were decided on the basis of significant changes in D-type stomata, typical stomata and cork-warts associated with leaf development. Epidermal scrapings from leaves at different development stages were made by excising several pieces of roughly 0.25 cm² from near the mid-blade. These epidermal scrapings were mounted in glycerin and examined under a LEICA DM LB2 fluorescent microscope (Leica Co., Wetzlar, Germany) with UV-light excitation and photographed using a Nikon coolpix 4500 digital camera (Nikon Co., Tokyo, Japan).

2.2 Leaf sectioning

For continuous paraffin sectioning, samples were cut into 10 µm pieces, and fixed in FAA solution. The leaf material was dehydrated in a graded ethanol series, embedded in paraffin, sectioned, mounted on glass slides, and stained with 0.5% hematoxylin solutions. The sections were examined and those at typical stages (corresponding to the above mentioned stages decided by fluorescence microscopy) were photographed using an OLYMPUS BX50 light microscope (Olympus Co., Tokyo, Japan).

2.3 Gas exchange and water evaporation measurements

Gas exchange measurements were taken on fully expanded adult and fully expanded youngest leaves (corresponding to two of the above mentioned stages, respectively) of *C. japonica* (with D-type stomata and cork-warts) using Portable Gas Exchange System GFS-3000 (Heinz Walz GmbH Co., Effeltrich, Germany). Parallel runs were conducted with fully expanded adult leaves and fully expanded youngest leaves of *C. reticulata* (without D-stomata and cork-warts). The leaf temperature was

set to 30°C and the CO₂ concentration at 340 ppm. Changes in net photosynthetic rates, water evaporation rates, and stomatal conductance were continuously monitored. It took approximately 30–60 min for the gas exchange parameters to reach their stable values. A red–blue light source (LED Light Source 3040-L) under software control was mounted on the leaf chamber as the source of light. The changes of stomatal conductance and evaporation rates were obtained under the photosynthetic active radiation (PAR) values of 1200, 1000, 800, 600, 400, 300, 200, 100, 50 and 0 mmol m⁻² s⁻¹, respectively. Leaves were allowed to stabilize at each light step for a minimum of 4 min and then data were logged after stable conditions were achieved. From each curve, the measurements of stomatal conductance and water evaporation rates of the leaf photosynthetic response to PAR were obtained and averaged for replicates of three leaves (Mao et al., 2007). Finally, the differences of stomatal conductance and water evaporation rates between *C. japonica* and *C. reticulata* were assessed.

2.4 Pressure experiment

The pressure experiment was conducted in a penicillin bottle. A fresh adult leaf of *C. japonica* (with cork-warts) was excised from plants and then pushed through a rubber stopper immediately. The penicillin bottle was filled with two-thirds volume of water and sealed with a rubber stopper, leaving the petiole outside and the lamina inside the bottle. A syringe was used to carefully draw some air out of the bottle to create negative pressure. As the air pressure inside the bottle was steadily lowered, some air bubbles appeared on the cork-warts. The leaf was then photographed using a Moticam 2000 digital camera mounted on a Motic K-400L stereo microscope (Motic China Group Co., Xiamen, China).

2.5 FTIR micro-spectroscopy analysis

FTIR spectroscopy provides a powerful and rapid assay for cell wall components and putative cross-links by identifying polymers and functional groups nondestructively. The availability of micro-sampling and data acquisition techniques that permit subtraction of the blanket absorption of water makes FTIR spectroscopy particularly suitable for studies of cell wall architecture (McCann et al., 1992). To determine if the cork-wart accumulated suberin, FTIR micro-spectroscopy analysis was carried out to compare the cell wall composition among CWP, mature typical stomata and cork-warts. The method was followed Wang et al. (2003; 2005) and Lu et al. (2004) with some modifications. After fixing for 24h, the leaf samples were dried in a layer on a barium fluoride window. Spectra were analyzed in D-type stomata with functions, cork-warts and typical stomata with functions by a MAGNA 750 FTIR spectrometer (Nicolet Corporation, Tokyo, Japan) equipped with a

mercury-cadmium-telluride detector. The spectra were recorded at a resolution of 8 cm^{-1} with 128 co-added interferograms and were normalized to obtain relative absorbance.

3. Results

3.1 Ontogenesis of D-type stomata and cork-warts in six different developmental stages

The six different developmental stages of D-type stomata and cork-warts in the leaf abaxial epidermis of a hypostomatic *C. japonica* are shown in Fig. 1. Initially, at the leaf bud stage, the original D-type stomata were only visible as some bright spots on the epidermis (Fig. 1A). At the second stage, before leaf expansion, each D-type stomata consisted of two guard cells and was morphologically similar to typical stomata (Fig. 1B). At stage three, partly expanded leaves, the D-type stomata started to have air-exchange functions associated with leaf expansion (Fig. 1C). At the next stage, when leaves had fully expanded, the large but infrequently appearing D-type stomata developed to adult stage while the tender, typical stomata appeared gradually (Fig. 1D). At the fifth stage when the D-type stomata started to decay, the well developed typical stomata densely covered the entire leaf abaxial epidermis (Fig. 1E). From this stage on, the CWP beside the guard cells (GCs) of D-type stomata showed strong signs of cell division. At the sixth stage, three main types of cork-warts were well developed and started their air-exchange function (Type I, II and III refer to Fig. 1F, G and H). Type I and II still had remnant D-type stomata structures, but the orifices were fully filled in Type II (Fig. 1G). However, in Type III, the GCs and subsidiary cells in the surface layer had fully degenerated and suberized (Fig. 1H). The number of smaller, typical stomata was usually 8 to 20 times of the D-type stomata or cork-warts, but their size was only about one fourth of a D-type stomata or one ninth of an entire cork-wart area.

The fluorescence character of D-type stomata showed that the chemical compounds changed gradually in typical stomata and cork-warts throughout their development. Under UV excitation, the ventral walls of the GCs of D-type stomata changed from yellowish green to blue-violet (Fig. 1B-E), which was associated with a similar fluorescent change of mesophyll cells.

Cross-sections of D-type stomata and cork-warts at different developmental stages showed that at the earliest stages of ontogenesis, there was nearly no orifice, but two connective guard cells could be seen (Fig. 2A, B). At the second stage, the CWP beside the subsidiary cells of the D-type stomata had appeared (arrow) in unexpanded leaves (Fig. 2C, D). Cells of the CWP were usually smaller than any of their neighbors and all were flattened thin-walled. At the next stage, the cells of the CWP became evident and started to divide (arrow), which was associated with leaf

expansion (Fig. 2E, F). After the third stage, another CWP (arrow), besides the guard cells of the D-type stomata, started to divide (Fig. 2G, H). As the CWP around the D-type stomata proliferated, a cluster of cells of spongy parenchyma formed an internal cavity (arrow) which was in contact with a D-type stoma (Fig. 2I). The CWP around the D-type stomata consisted of thin-walled cells with a dense cytoplasm. Later, the CWP stopped dividing and different types of cork-warts were formed (Fig. 2J, K, as there was no significant difference found between paraffin sections of Type I and Type III, only two figures are shown here). As the cavity expanded, the cells of the spongy parenchyma matured or degenerated, simultaneously.

3.2 Gas exchange and water evaporation

The light respond curves of transpiration rate and stomatal conductance showed similar trends in *C. japonica* and *C. reticulata*, except that *C. japonica* plants which had D-type stomata showed higher values of transpiration compared to *C. reticulata* plants without D-type stomata (Fig. 3, A, B). In *C. japonica*, two patterns of transpiration responses were evident, with the curve of the young leaf showing a double-peak. One peak occurred in the light intensity range from 0 $\text{mmol m}^{-2} \text{s}^{-1}$ to 600 $\text{mmol m}^{-2} \text{s}^{-1}$ and the other peak occurred in the light intensity range above 600 $\text{mmol m}^{-2} \text{s}^{-1}$. The transpiration response curve of adult leaves displayed a single-peak curve. The light saturation point occurred at about 800 $\text{mmol m}^{-2} \text{s}^{-1}$. However, both young and adult leaves of *C. reticulata* exhibited a single-peak curve but with a light saturation point at 800 $\text{mmol m}^{-2} \text{s}^{-1}$, similar to *C. japonica*.

Camellia japonica plants also showed higher values of stomatal conductance than *C. reticulata* plants, and had two patterns of stomatal conductance responses. One peak of young leaves occurred in the light intensity range from 0 $\text{mmol m}^{-2} \text{s}^{-1}$ to 600 $\text{mmol m}^{-2} \text{s}^{-1}$ and the other peak occurred in the light intensity range above 600 $\text{mmol m}^{-2} \text{s}^{-1}$. The stomatal conductance response curve of adult leaves showed a single-peak curve. But both young and adult leaves of *C. reticulata* showed a single-peak curve but with a light saturation point at 800 $\text{mmol m}^{-2} \text{s}^{-1}$.

3.3 Pressure experiment and gas exchange

The pressure experiment showed that many air bubbles (Fig. 4A) occurred on the cork-warts scattered across the leaf surface of *C. japonica* when the air pressure was lowered. Just a few small air bubbles appeared in other areas of the leaf lacking cork-warts (data not shown). In comparison, there was no air bubbles observed on the leaf surface of *C. reticulata* in this experiment. Leaf cross-sections showed that, in spite of multiple mesophyll cells arranged peripherally, there were still some interspaces going through them into the spongy tissue (Fig. 4B). This indicates cork-warts could potentially facilitate gas exchange in *C. japonica*.

3.4 FTIR micro-spectroscopy

FTIR micro-spectroscopy results of matured typical stomata, cork-warts, and digital subtraction between cork-warts and matured typical stomata are shown in Fig. 5. In plant material, peaks at 2920 cm^{-1} (C–H aliphatic stretch), 2856 cm^{-1} (C–H aliphatic stretch) and 1735 cm^{-1} (C=O stretch, ester group) are used to monitor suberin (Rocha et al., 2001); the peak at 1608 cm^{-1} (C=C stretch, aromatic ring) to monitor the phenolic polymer in suberin (Marques et al., 1999; Rocha et al., 2001); peaks at 1277 cm^{-1} to monitor the typical CC stretching vibration bands in the ester (Rocha et al., 2001; Ming et al., 2002); the peak at 1466 to monitor the pectic substances (Jin et al., 2005); the peak at 1430 to monitor the semi-cellulose (Sun et al., 2007); the peak at 1190 cm^{-1} to monitor C=O stretch of cell wall fructose (Kelly and Downey, 2005).

Compared to matured typical stomata, cork-warts accumulated more suberin (2920 cm^{-1} , 2856 cm^{-1} and 1735 cm^{-1}), ester (1277 cm^{-1}), cell wall fructose (1190 cm^{-1}), and cell wall polysaccharides such as pectic substances (1466 cm^{-1}) (Fig. 5C).

4. Discussion

Cork-warts observed in *C. japonica* are stomata-type warts, since they originated from the tangential divisions in the peripheral cells of D-type stomata. The same cork-wart type has been reported in *Ilex* (Korn and Fredrick, 1973), and *Celastrus*, *Goniodiscus*, *Maytenus* and *Plenckia* (Joffily and Cardoso Vieira, 2010). Our ontogenesis results supported Korn and Fredrick's view (1973) that large D-type stomata and cork-warts occur at the same location. The paradermal section results indicated when D-type stomata started to suberize, the CWP beside the GCs of D-type stomata had robust division ability. Similarly, Korn and Fredrick (1973) demonstrated that D-type stomata were developmental structures which were arranged in an ordered pattern over the leaf surface. In other words, the D-type stomata represented the early stage of cork-warts. They have the same origin but are structures with different functions at different developmental stages of the leaf. Here, our research further indicated that cork-warts originate late from CWP around the D-type stomata, after most of the typical stomata were well developed and functional. Our cross section results also support that D-type stomata are relatively short-lived compared to typical leaf stomata observed by Korn and Fredrick (1973). Moreover, it showed that D-type stomata emerge earlier on the leaves than typical stomata (Fig. 1A, B, C, D) and open at higher light intensities.

Joffily and Cardoso Vieira (2010) considered that cork-warts may be related to the “preinfectious structural defence” as proposed by Dickison (2000). The suberization during the formation of cork-warts may serve as “a resistant barrier against infections and/or provokes the death of infected cells”, and that this repair process may be

triggered by random injury or insect punctures (Haberlandt, 1914; Stace, 1965). However, the presence of cork-warts on the abaxial leaf epidermis of *Camellia* seemed to be a common physiological process as they persistently occur in certain species and certain taxonomic sections in this genus (Jiang et al., 2010). Recently, Evans and Bromberg (2010) reported that matured cork-warts may have function of internal airflow in red mangrove species (*Rhizophora*), providing gas exchange from leave to the anoxic substrate through internally connected aerenchyma. Our pressure experiment here also indicate that cork-warts on *Camellia* leaves could potentially facilitate gas exchange. Gas exchange and water evaporation measurements further suggests *C. japonica* (with cork-warts) had a higher transpiration rate stomatal conductance compared to *C. reticulata* plants (without cork-warts) (Fig. 3 A & B).

Through investigation of *Camellia* species and their ecological habitat, we found that the majority of *Camellia* species (39 of 43, 90.7%, Table S1) with cork-warts tend to occur as understory trees in evergreen forests, and at high humidity levels (84.4%, Table S2). Since cork-warts can be considered “nonrestrictive water evaporation vent holes”, leaves with cork-warts can be more sensitive to the surrounding humidity and temperature than species without cork-warts. Therefore, whether cork-warts could be a physiological response to a humid environment becomes an interesting hypothesis. Although further testing of this hypothesis is needed, but a possible explanation would be an effective water evaporation reducing the leaf temperature. Cork-warts might have evolved as an organ to help plants adapt to humid environments. As pros and cons, this could also lead to the lack of resistance to cold environments. Indeed, the majority of *Camellia* species with cork-warts are cold sensitive (76.92%, Table S3), and none of the *Camellia* species occurring at higher altitudes possess cork-warts (Table S4). Knowledge of the characteristics of stomatal transpiration may help to assess the ability of plants to withstand high or low humidity conditions with regard to plant adaptation. The presence of warts seems to be an obstacle for plants growing in open habitats, as the available ecological data for *Camellia* shows that most species (90.9%) occurring in open habitats have no warts, and nearly half of the understory species possess cork-warts (Table S1). There is no doubt that possessing a good water evaporator, and hence allowing a plant to regulate water evaporation through variation of its stomatal conductance, represents the main physiological role of the plant epidermis (Kerstiens, 2006).

5. Conclusion

The results of the current study show that (1) the large D-type stomata and cork-warts in *Camellia* are two functional structures at different developmental stages and originate from the adjacent protomeristems, (2) the large D-type stomata and cork-warts on the leaf epidermis may have important roles in the regulation of water

evaporation and gas exchange, 3) the presence of large D-type stomata and cork-warts possibly correlates with the adaptation of *Camellia* species to more humid understory environment.

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References:

- Chang, H.T., 1996. Diagnosis on the systematic development of Theaceae VII The taxonomic problem of Section *Glaberrima* Chang of *Camellia*. Acta Sci. Nat. Univ. Sunyatseni 35, 88 – 91.
- Chang, H.T., 1998, *Camellia*. In: Chang, H.T. (Ed.), Flora Reipublicae Popularis Sinicae. Science Press, Beijing.
- Dickison, W.C., 2000, Integrative plant anatomy. Academic Press.
- Evans, L.S., Bromberg, A., 2010. Characterization of cork warts and aerenchyma in leaves of *Rhizophora mangle* and *Rhizophora racemosa*. J.Torrey Bot. Soc. 137, 30 – 38.
- Evans, L.S., Okawa, Y., Searcy, D.G., 2005. Anatomy and morphology of red mangrove (*Rhizophora mangle*) plants in relation to internal airflow. J.Torrey Bot. Soc. 132, 537 – 550.
- Farooqui, P., 1982. Cork-warts in *Eucalyptus* species. Proceedings: Plant Sciences 91, 289 – 295.
- Gao, J.Y., Parks, C.R., Du, Y.Q., 2005, Collected species of the genus *Camellia*, an illustrated outline. Zhejiang Technology and Science Press, Hangzhou.
- Haberlandt, G., 1914, Physiological plant anatomy. Macmillan, London.
- Jiang, B., Peng, Q.F., Shen, Z.G., Möller, M., Pi, E.X., Lu, H.F., 2010. Taxonomic treatments of *Camellia* (Theaceae) species with secretory structures based on integrated leaf characters. Plant Syst. Evol. 290, 1 – 20.
- Jin, X., Pu, J., Xie, Y., Takeshi, F., Liu, X., 2005. Lignin characterization of triploid clones of *Populus tomentosa* Carr. Forestry Studies in China 7, 52 – 56.
- Joffily, A., Cardoso Vieira, R., 2010. Cork-warts on the leaf epidermis of four genera of Celastroidea-Celastraceae. Flora 205, 313 – 318.
- Kelly, J.D., Downey, G., 2005. Detection of sugar adulterants in apple juice using Fourier transform infrared spectroscopy and chemometrics. J. Agr. Food Chem. 53, 3281 – 3286.
- Keng, H., 1962. Comparative morphological studies in Theaceae. Univ. Calif. Publ. Bot. 33, 269 – 384.
- Kerstiens, G., 2006. Water transport in plant cuticles: an update. J. Exp. Bot. 57, 2493 – 2499.

- Korn, R.W., Fredrick, G.W., 1973. Development of D-type Stomata in the Leaves of *Ilex crenata* var. *convexa*. Ann. Bot.-London 37, 647 – 656.
- Lu, H.F., Cheng, C., Tang, X., Hu, Z., 2004. FTIR spectrum of *Hypericum* and *Triadenum* with reference to their identification. Acta Bot. Sin. 46, 401 – 406.
- Mao, L.Z., Lu, H.F., Wang, Q., Cai, M.M., 2007. Comparative photosynthesis characteristics of *Calycanthus chinensis* and *Chimonanthus praecox*. Photosynthetica 45, 601 – 605.
- Marques, A., Pereira, H., Meier, D., Faix, O., 1999. Structural characterization of cork lignin by thiadidolysis and permanganate oxidation. Holzforschung 53, 167 – 174.
- Matteucci, E., 1897. Contributo allo studio delle placche sugherose nelle piante. Nuovo Giorn. Bot. Ital. 4, 224 – 244.
- McCann, M.C., Hammouri, M., Wilson, R., Belton, P., Roberts, K., 1992. Fourier transform infrared microspectroscopy is a new way to look at plant cell walls. Plant Physiol. 100, 1940 – 1947.
- Metcalfe, C.R., Chalk, L., 1950, Anatomy of the Dicotyledons. Clarendon Press, Oxford.
- Ming, T.L., 2000, Monograph of the genus *Camellia*. Yunnan Science and Technology Press, Kunming.
- Ming, W., Tian, M., Van de Grampel, R.D., Melis, F., Jia, X., Loos, J., Van der Linde, R., 2002. Low surface energy polymeric films from solventless liquid oligoesters and partially fluorinated isocyanates. Macromolecules 35, 6920 – 6929.
- Parks, C.R., Griffiths, A., 1963. The saluenensis-pitardii-reticulata complex. Camellia Rev. 25, 12 – 29.
- Poulsen, V.A., 1875. Om Korkdannelse paa Blade. Vid. Med. Dansk Nat. For. Kjøbenhavn 37, 44 – 58.
- Rocha, S.M., Goodfellow, B.J., Delgadillo, I., Neto, C.P., Gil, A.M., 2001. Enzymatic isolation and structural characterisation of polymeric suberin of cork from *Quercus suber* L. Int. J. Biol. Macromol. 28, 107 – 119.
- Sealy, J.R., 1958, A Revision of the Genus *Camellia*. The Royal Horticultural Society, London.
- Shen, J., Lu, H., Peng, Q., Zheng, J., Tian, Y., 2008. FTIR spectra of *Camellia* sect. *Oleifera*, sect. *Paracamellia*, and sect. *Camellia* (Theaceae) with reference to their taxonomic significance. J. Syst. Evol. 46, 194 – 204.
- Stace, C.A., 1965. Cuticular studies as an aid to plant taxonomy. Bull. Brit. Mus. (Nat. Hist.), Bot. 4, 3 – 78.
- Sun, Y., Lin, L., Pang, C., Deng, H., Peng, H., Li, J., He, B., Liu, S., 2007. Hydrolysis of cotton fiber cellulose in formic acid. Energ. Fuel. 21, 2386 – 2389.
- Wang, Q., Kong, L., Hao, H., Wang, X., Lin, J., Šamaj, J., Baluška, F., 2005. Effects of brefeldin A on pollen germination and tube growth. Antagonistic effects on endocytosis and secretion. Plant Physiol. 139, 1692 – 1703.
- Wang, Q., Lu, L., Wu, X., Li, Y., Lin, J., 2003. Boron influences pollen germination and pollen tube growth in *Picea meyeri*. Tree Physiol. 23, 345 – 351.