BIOLOGIA PLANTARUM 55 (4): 716-720, 2011

BRIEF COMMUNICATION

# Guard cells on adaxial and abaxial epidermes of *Erythrina corallodendron* sepals

B. HUANG[1], Y. WANG[1], S. HUANG[2] and S. MA[1]*

*College of Life Science and Technology[1] and College of Medicine[2], Jinan University, Guangzhou 510632, P.R. China*

## Abstract

This study investigated guard cells on the adaxial and the abaxial epidermes during *Erythrina corallodendron* sepal development. On the adaxial epidermis, the morphology of guard cells was highly variable and changes in aperture induced by abscisic acid (ABA) were observed in 9.1 % stomata, while on the abaxial epidermis 86.7 % stomata responded to ABA. On the adaxial epidermis, stomata did not close even when guard cell pressure potential was reduced to zero by plasmolysis, even if fluorescein diacetate revealed that guard cells were alive. It was concluded that guard cells on the adaxial and the abaxial epidermes of sepals sensed environmental conditions differently, maybe due to different guard cell wall elasticity.

*Additional key words*: abscisic acid, cell wall elasticity, plasmolysis, stomatal movements.

A flowering shrub *Erythrina corrallodendron* L. (*Leguminosae*) is distributed in tropical and subtropical regions worldwide. Guard cell walls, due to their unique properties and non-uniform thickness, are of fundamental importance for the stomatal movements (Aylor *et al*. 1973, Christodoulakis *et al*. 2002). Stomatal movement is achieved through changes in guard-cell volume and pressure potential. Among plant hormones, abscisic acid (ABA) inhibits stomatal opening and induces stomatal closure. The mechanisms of stomata action have been reviewed many times (*e.g.* Raschke and Hedrich 1985, Blatt 2000, Roelfsema and Hedrich 2002, Wang and Song 2008).

Apart from being mainly presented on the leaves, stomata are also observed in other plant organs including sepals. Occurrence of $CO_2$ uptake by sepals, regulated by their stomata, is regarded as an important strategy of additional carbon acquisition for flowers (Dueker and Arditti 1968, Aschan and Pfanz 2003, Aschan *et al*. 2005). However, to our knowledge, there is little information available about whether guard cells on adaxial and abaxial epidermes of sepals respond similarly to environmental conditions as leaf stomata. Therefore, this study was mainly focused on changes in guard cells on the adaxial and the abaxial epidermis of sepals during *E. corallodendron* flower development.

*Erythrina corrallodendron* L. plants were grown under natural conditions in the Garden of Jinan University, P.R. China. In order to eliminate the differences between plants in different flower developmental stages, flowers were randomly selected from only one plant. When this plant flowered, flower development was divided into six stages which were based on the length of outermost petals (Fig. 1*A*).

At each stage, the middle area of adaxial and abaxial epidermes of sepals, after thorough washing in the running water, were peeled off. The clean strips were immediately mounted on slides for observing the morphology of stomata under a *Nikon YS100* (Japan) light microscope under 400 × magnification.

The other clean strips were immediately submerged in $K^+$-MES buffer. KCl (50 mM) was then added to induce stomatal opening completely. The average stomatal aperture was measured on about 200 stomata before and

*Received* 26 February 2010, *accepted* 5 January 2011.
*Abbreviations*: ABA - abscisic acid; MES - 2-(*N*-morpholino) ethanesulfonic acid.
*Acknowledgements*: We thank Dr. Fangli Lu for critical reading and revision in writing.
* Corresponding author; fax: (+86)02038897606, e-mail: msmwdw@163.com

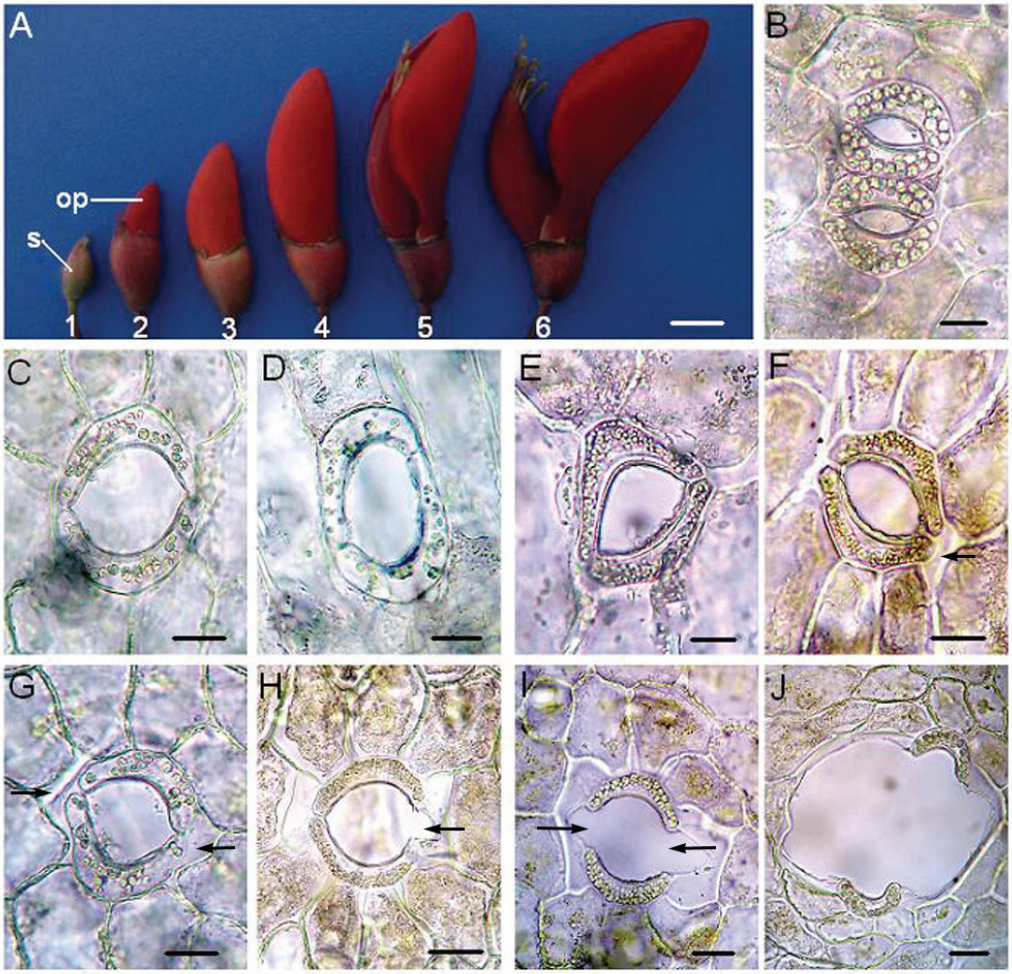


Fig. 1. The developmental stages of *E. corallodendron* flowers and the structure of stomata on the adaxial epidermis of sepals: *A* - flower development was divided into 6 stages which were based on the length of outermost petals (1, 2, 3, 4, 5 and 6 cm); *B* - two stomata were in direct contact; *C* - a circular-like stoma; *D* - a rectangular-like stoma; *E* - triangular-like stoma; *F* - a fissure was formed at the junction between one pole of stoma and the neighboring epidermal cells (*arrow*); *G* - two fissures were formed at the junction between two poles of stoma and the neighboring epidermal cells (*arrows*); *H* - two guard cells were split at one pole of stoma (*arrow*); *I* - two guard cells were split at the two poles of stoma (*arrows*); *J* - a large opening was produced (s - sepal, op - outmost petal, *bar A* = 1.0 cm, *B – J* = 20 μm).

after $10^{-4}$, $10^{-3}$, $10^{-2}$, $10^{-1}$ or 1 mM ABA was added to the $K^+$-MES butter, and the effect of ABA on stomatal aperture (Δ) was calculated as follows: $\Delta = [(R_b - R_a)/R_b] \times 100$ %, where $R_b$ and $R_a$ is the average width of stomatal aperture before and after the ABA treatment. In order to check whether guard cells underwent plasmolysis on the abaxial and the adaxial epidermes of sepals, the clean strips from each stage were submerged in sucrose solution (1.0 M) for 0.5 h. Before making the plasmolysis of guard cells, these clean strips were submerged in the flurescein diacetate solution (0.3 g $dm^{-3}$) for 10 min in order to check whether guard cells were alive. About 50 stomata per stage were recorded using a *Leica DMI 4000B* (Germany) fluorescence microscope equipped with a digital camera. The experiment was repeated three times.

We observed that stomatal formation took place when sepals emerged in the flowers, and stomata were evenly distributed on the adaxial epidermis. In addition to normal stomata, commonly separated by a number of epidermal cells, stomatal clusters were also observed on the adaxial epidermis (Fig. 1*B*). The morphology of guard cells was highly variable during sepal development. In addition to oval stomata, circular, rectangular, and triangular-like stomata were observed (Fig. 1*C*,*D*,*E*). Some stomata continued to degenerate, leaving large openings. When the stomatal aperture became abnormally large, fissures were formed at the junctions between poles of stoma and the neighboring epidermal cells (Fig. 1*F*,*G*). Further, two guard cells were split at poles of stoma (Fig. 1*H*,*I*). 'Half' of the stoma, composed of one guard

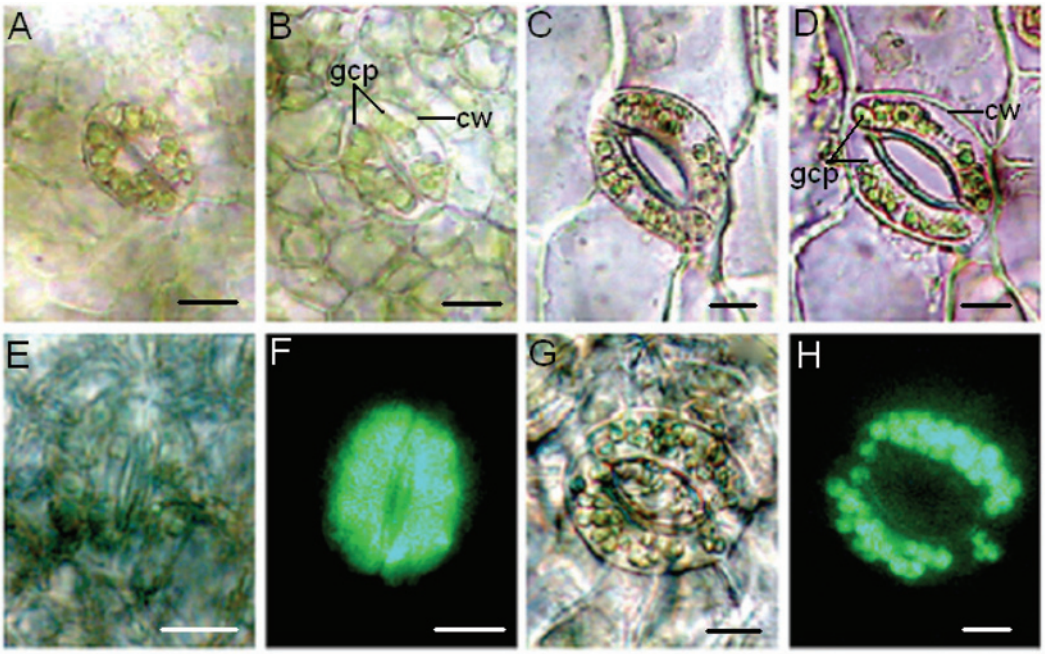


Fig. 2. Stomatal response to sucrose under light microscope or to fluorescein diacetate under flurescence microscope. A close stoma from the abaxial epidermis of sepal (*A*). A plasmolyzed and closed stoma from the abaxial epidermis (*B*). An open stoma from the adaxial epidermis of sepal (*C*). A plasmolyzed but open stoma from the adaxial epidermis of sepal (*D*). A stoma from the abaxial epidermis of sepal (*E*). A fluoresced stoma from the abaxial epidermis (*F*). A stoma from the adaxial epidermis of sepal (*G*). A fluoresced stoma from the adaxial epidermis of sepal (*H*). gcp - guard cell protoplast, cw - cell wall. *Scale bar* = 20 μm.

Table 1. The effects of ABA on aperture of stomata on the adaxial and abaxial epidermes of *E. corallodendron* sepals. The stomatal aperture was measured before ($R_b$) and after ABA ($R_a$) was added to the $K^+$-MES butter. The effect of different concentrations ($10^{-7}$, $10^{-6}$, $10^{-5}$, $10^{-4}$, and $10^{-3}$ M) was measured at stage 1 and incubation time 3.0 h. The effect of incubation time (1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 h) was measured at $10^{-4}$ M ABA and also at stage 1. The effect of different stages from 1 to 6 (see Fig. 1*A*) was measured at $10^{-4}$ M ABA and incubation 3.0 h. Means ± SE, $n$ = 200.

| ABA treatments | | Adaxial epidermis $R_b$ [μm] | $R_a$ [μm] | Δ [%] | Abaxial epidermis $R_b$ [μm] | $R_a$ [μm] | Δ [%] |
|---|---|---|---|---|---|---|---|
| Concentrations [M] | $10^{-7}$ | 13.9 ± 5.8 | 13.4 ± 5.1 | 3.8 | 7.5 ± 1.3 | 2.7 ± 0.5 | 64.0 |
| | $10^{-6}$ | 12.9 ± 5.2 | 12.5 ± 5.1 | 3.0 | 7.0 ± 1.0 | 0 | 100 |
| | $10^{-5}$ | 12.9 ± 6.7 | 12.0 ± 6.3 | 7.0 | 7.8 ± 1.5 | 0 | 100 |
| | $10^{-4}$ | 11.3 ± 5.9 | 10.0 ± 5.0 | 11.2 | 8.1 ± 0.9 | 0 | 100 |
| | $10^{-3}$ | 11.8 ± 5.5 | 11.0 ± 5.7 | 6.2 | 6.9 ± 1.1 | 0 | 100 |
| Incubation [h] | 1.0 | 12.3 ± 7.2 | 12.2 ± 7.3 | 1.1 | 7.5 ± 1.2 | 1.6 ± 0.6 | 78.9 |
| | 2.0 | 13.2 ± 7.0 | 13.0 ± 7.7 | 2.1 | 8.0 ± 1.0 | 0 | 100 |
| | 3.0 | 15.1 ± 9.6 | 14.2 ± 8.4 | 5.7 | 7.5 ± 0.8 | 0 | 100 |
| | 4.0 | 15.4 ± 7.8 | 14.6 ± 7.3 | 5.6 | 8.5 ± 1.4 | 0 | 100 |
| | 5.0 | 13.8 ± 6.1 | 12.2 ± 6.5 | 12.0 | 8.1 ± 1.5 | 0 | 100 |
| | 6.0 | 12.5 ± 5.8 | 11.2 ± 5.2 | 10.1 | 7.7 ± 0.6 | 0 | 100 |
| Sepal stages | 1 | 14.3 ± 7.2 | 13.0 ± 6.7 | 9.1 | 8.8 ± 0.9 | 0 | 100 |
| | 2 | 13.8 ± 7.0 | 13.2 ± 6.1 | 4.5 | 8.2 ± 1.4 | 0 | 100 |
| | 3 | 15.2 ± 7.2 | 14.9 ± 6.9 | 2.3 | 7.5 ± 0.5 | 1.0 ± 0.4 | 86.7 |
| | 4 | 16.3 ± 9.4 | 15.2 ± 9.0 | 6.6 | 8.5 ± 0.6 | 0.8 ± 0.3 | 90.6 |
| | 5 | 18.4 ± 7.4 | 16.8 ± 6.6 | 8.7 | 9.1 ± 0.8 | 0.8 ± 0.1 | 91.2 |
| | 6 | 15.0 ± 7.6 | 14.2 ± 7.6 | 5.2 | 8.7 ± 1.1 | 0 | 100 |

cell, moved apart from the other 'half'. During the degeneration of neighboring epidermal cells, a large opening was formed eventually in the adaxial epidermis (Fig. 1*J*).

In the present study, large openings by stomata were found in the adaxial epidermis of *E. corallodendron* sepals. To our knowledge, this is the first report about it in these plant organs, which needs to be interpreted. The formation of large openings in the adaxial epidermis can be explained only if the fate of stomata is followed carefully. In leaves, epidermal cells reduce stomatal aperture up to 50 % by pressing on the guard cell wall (Roelfsema and Hedrich 2002, 2005). Furthermore, the stomata of barley genotypes attacked by *Blumeria graminis* remained steadily open as a consequence of the death of epidermal cells (Prats *et al*. 2006). In the present study, fissures were formed at the junction between two poles of stoma and the neighboring epidermal cells. Due to the destructive junction region, the epidermal cells would no longer press against the guard cells and the stoma would continue to open. We also observed that two guard cells were split at the poles of stoma and a large opening was formed eventually as the fate of stoma. Our data indicate that the emergence of these fissures could be the first step in the process of formation of large openings in the adaxial epidermis of sepals. Similar openings by transverse disruption of stomata have been reported in the primary root of *Ceratonia siliqua* L., in which two halves of stomata move apart from the each (Christodoulakis *et al*. 2002). *Via* large openings in the adaxial epidermis of *E. corallodendron* sepals, a significant water loss and the desiccation of sepals may be produced.

In order to check whether guard cells on the adaxial and abaxial epidermes of sepals respond similarly to environmental conditions as leaf guard cells, we measured the effect of ABA at various concentrations and incubation time on stomatal aperture. At stage 1 of sepal development, decrease in aperture induced by ABA was only 11.2 to 12.0 % on adaxial epidermis, while stomata on the abaxial epidermis closed almost completely in response to ABA (Table 1). For all developmental stages, stomatal apertures on the adaxial epidermis decreased by 9.1 % after 3 h of $10^{-4}$ M ABA treatment. In contrast, 86.7 % change in stomatal aperture was induced on the abaxial epidermis. The similar non-functional stomata were also observed on other plant organs. In orchid flowers, stomata failed to respond to irradiance, water vapor deficit, $CO_2$ concentration, and ABA (Hew *et al*. 1980, Goh 1983). In *Populus* × *euramericana* subjected to Zn excess, impairments in leaf photosynthesis could be due to stomatal malfunctioning (Di Baccio *et al*. 2010). Our data showed that guard cells on the adaxial epidermis were not sensitive to environmental conditions during further stages of sepal development.

As stomatal movements are usually correlated with guard cell pressure potential and volume, a hypertonic solution was used. Stomata on the abaxial epidermis closed and guard cells were plasmolyzed after the epidermal strips were submerged in 1.0 M sucrose solution for 0.5 h (Fig. 2*A*,*B*), while stomata on the adaxial epidermis remained open even when their guard cells were plasmolyzed (Fig. 2*C*,*D*). When the adaxial and abaxial epidermal strips were submerged in the flurescein diacetate solution (0.3 g $dm^{-3}$) for 10 min, fluorescein diacetate revealed that guard cells from both epidermes remained alive (Fig. 2*E*-*H*).

The failure of stomata on the adaxial epidermis of *E. corallodendron* sepals to close may be caused by the low guard cell wall elasticity or the death of guard cells. The above mentioned results indicated that the failure of stomata to close may be mainly attributed to low cell wall elasticity, because the stomata remained open even when guard cell pressure potential was zero but the guard cell remained alive. This may also explain the smaller change in stomatal aperture in response to ABA on the adaxial epidermis.

In summary, stomata on the adaxial and abaxial epidermes of *E. corallodendron* sepals underwent different developmental changes and differently responded to environmental condition. Large openings on the adaxial epidermis may increase the diffusion of $CO_2$ inside flower but also wilting of sepals.

## References


Aschan, G., Pfanz, H.: Non-foliar photosynthesis - a strategy of additional carbon acquisition. - Flora **198**: 81-97, 2003.

Aschan, G., Pfanz, H., Vodnik, D., Batic, F.: Photosynthetic performance of vegetative and reproductive structures of green hellebore (*Helleborus viridis* L. agg.). - Photosynthetica **43**: 55-64, 2005.

Aylor, D.E., Parlange, J.-E., Krikorian, A.D.: Stomatal mechanics. - Amer. J. Bot. **60**: 163-171, 1973.

Blatt, M.R.: $Ca^{2+}$ signalling and control of guard-cell volume in stomatal movements. - Curr. Opin. Plant Biol. **3**: 196-204, 2000.

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

Di Baccio, D., Minnocci, A., Sebastiani, L.: Leaf structural modifications in *Populus* × *euramericana* subjected to Zn excess. - Biol. Plant. **54**: 502-508, 2010.

Dueker, J., Arditti, J.: Photosynthetic $^{14}CO_2$ fixation by green *Cymbidium* (Orchidaceae) flowers. - Plant Physiol. **43**: 130-132, 1968.

Goh, C.J.: Rhythms of acidity and $CO_2$ production in orchid flowers. - New Phytol. **93**: 25-32, 1983.

Hew, C.S., Lee, G.L., Wong, S.C.: Occurrence of non-functional stomata in the flowers of tropical Orchids. - Ann.

Bot. **46**: 195-201, 1980.

Prats, E., Gay, A.P., Mur, L.A.J., Thomas, B.J., Carver, T.L.W.: Stomatal lock-open, a consequence of epidermal cell death, follows transient suppression of stomatal opening in barley attacked by *Blumeria graminis*. - J. exp. Bot. **57**: 2211-2226, 2006.

Raschke, K., Hedrich, R.: Simultaneous and independent effects of abscisic acid on stomata and the photosynthetic apparatus in whole leaves. - Planta **163**: 105-118, 1985.

Roelfsema, M.R.G., Hedrich, R.: Studying guard cells in the intact plant: modulation of stomatal movement by apoplastic factors. - New Phytol. **153**: 425-431, 2002.

Roelfsema, M.R.G., Hedrich, R.: In the light of stomatal opening: new insights into 'the Watergate'. - New Phytol. **167**: 665-691, 2005.

Wang, P., Song, C.P.: Guard-cell signaling for hydrogen peroxide and abscisic acid. - New Phytol. **178**: 703-718, 2008.