Hydropassive evidence and effective factors in stomatal oscillations of *Glycyrrhiza inflata* under desert conditions

Gen-Xuan Wang *, Jun Zhang, Jian-Xong Liao, Jian-Lin Wang

School of Biology, State Key Laboratory of Arid Agroecology, Lanzhou University, Lanzhou 730000, PR China

Received 19 August 1999; received in revised form 13 December 1999; accepted 10 January 2001

Abstract

Whether stomata oscillations induced by atmospheric drought stress are hydropassive or metabolic energy-dependent and the thresholds of some effective factors were studied in *Glycyrrhiza inflata*. The metabolic inhibitors NaN₃ and carbonyl cyanide-m-phenyl-hydrazone inhibited the respiration. However they could not significantly change the intensity (amplitude/average) of the stomata oscillations. The leaf turgor-pressure was fluctuated simultaneously with the stomata oscillations, whereas the K⁺ content of the guard cells did not show oscillations. The oscillation intensity was found to be regulated by vapour pressure deficit (VPD), the proportion of the retained root and the stem flux lag. The minimum threshold of VPD, roots and the stem flux lag may be required to induce the stomata oscillations. The fluctuations of the leaf turgor-pressure induced by the non-synchronization between the transpiration demand and provide of water by the stem flux may be the direct cause of the stomata oscillations in *G. inflata* under the conditions of high transpiration demand. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Stomata oscillation; Hydropassive movement; Leaf conductance; Vapour pressure deficit; Stem flux

1. Introduction

The concept of hydropassive movement of stomata was described by F. Darwin [1]. Stålfelt [2] further developed the terms 'hydropassive' and 'hydroactive' to describe the effects of tissue and cell water relations on stomatal movements. Hydropassive effects are resulted from the water availability in tissue which changes the turgor relations of the stomata complexes, whereas hydroactive effects are those that influence stomata movements via metabolic processes of the guard cells. Schulze et al. [3] have provided indirectly evidence of hydropassive movement of stomata by showing that a transient decline in transpiration rate near mid-day occurs when the lag of water absorption is sufficient to create a water deficit and result in temporary stomata closure. However, many results of recent reports [4–8] in the experiments using epidermal strips were not consistent with the concept of hydropassive movement of stomata. The uncoupler carbonyl cyanide-m-phenyl-hydrazone (CCCP) which short-circuits all pH-gradients, abolishing membrane potentials and photophosphorylation [4] was used to non-specifically reduce the metabolism and ATP-levels in the guard cells [5]. ABA-induced stomata closure was showed to be blocked by the metabolic inhibitor NaN₃ [8]. Thus, stomata movement induced by light [6,7] as well as ABA [8] appears a metabolic energy-dependent process. However, it had been documented that the initial humidity-dependent stomata movements were hydropassive in *Valerianella locusta*, followed by a second process with a lag of about 20 min [9] which control stomata movement through metabolic adjustment of guard cell turgor by changing guard cell K⁺ levels.

Stomata oscillations have been reported in more than 30 plant species [10–18]. Short period oscillations (less than 10 min) appear to be dependent on
external carbon dioxide concentration, while slower oscillations (30–50 min) with larger amplitude are associated with plant water status [10,19]. Several models, which incorporate the ideas of negative or positive feedbacks of leaf water status have been proposed to explain stomatal oscillations [14,20,15]. However the Resistance-Inductance-Capacitance (RLC) model which did not include the metabolic energy factors seems to be most suitable in explaining the stomatal oscillations of *Glycyrrhiza inflata* under atmospheric drought conditions [18]. It was hypothesized that the main energy source of stomatal movement are different between oscillations induced by high vapour pressure deficit (VPD) and that in steady state, the former is hydropassive and the later is metabolic dependant. The aim of this study is to examine whether stomatal oscillations induced by high VPD are hydropassive or metabolic energy-dependent and to comprehend the thresholds of its effective factors in *G. inflata*.

## 2. Materials and methods

### 2.1. Plant material and growing conditions

Perennial C₃ herb, *G. inflata*, was grown near the Dunhuang oasis of arid desert in Anxi county (40.5°N, 95.7°E) of Gansu province in the northwest of China. There are feather-like compound leaves, which adult leaf is about 3 cm wide and 6 cm length, in the plant. The roots of the plant are huge and much used in medicin. In this area, the mean of annual precipitation is 100 ± 20 mm. Although the surface layer of sand is very dry, there is enough moisture for the root absorption in the depth of 1–2 m where roots distributed. The water content varied between 10% and 15% by weight in the soil at the depth of 1 m. During the experiment period from the 8 July to the 10 August in 1998, the VPD in the air at plant canopy level varied from about 0 at dawn to 20–40 mPa Pa⁻¹ at 2–3 pm. The atmospheric temperature varied from 15 ± 4°C at dawn to 34 ± 5°C at 2–3 pm.

### 2.2. Xylem injection

The solution of inhibitors (0.1M NaN₃ or 1.0 mM CCCP in water) was injected into the plant through the xylem. The bottle filled with the injected solution was suspended above the plant. The syringe needle (0.4 mm in diameter) was inserted into the conducting xylem (20 ± 4 mm in diameter). Effective concentrations of the inhibitors in the tissues were adjusted by regulating the injection rate and the duration. The rate of injection was about 2 ml min⁻¹ in the experimental conditions. The xylem injection method could overcome the difficulties of getting the compounds across cuticles, and was easy to use in the field conditions in situ. At least eight independent measurements were conducted to calculate the mean value and standard deviations. The 10 mM azide (NaN₃) and 10 μM CCCP was used to treat the leaves separately based on the effective concentrations used by Karlsson and Schwartz in 1998 [5].

### 2.3. Measurement of eco-physiological index

Leaf conductance, temperature, relative humidity, transpiration and CO₂-exchange were measured in an open analysing system of photosynthesis [21] (H. Walz, Mess-und Regeltechnik, Germany). The leaf respiration rate was measured in the darkened leaf cuvette, which was wrapped by the silver paper. The values were recorded 5 min after the dark treatment. The relative metabolic rate was calculated as the ratio of respiration rate of treated plants to that of untreated ones.

The ratio of amplitude to the average value was defined as the intensity of the oscillation which served as a parameter for comparing the effects of different factors. Where, amplitude was the distance between the maximum (or minimum) and the average. Actually, the same value of amplitude may imply the different relative change if the average and/or period are different. The amplitude, average and period was calculated within a cycle when they all are changing. The effect of VPD on the intensity of stomata oscillations was estimated from the data of the eight duplicates of the diurnal dynamics of leaf conductance in the adult leaves. The VPD were estimated as the corresponding value at the middle time of the cycle.

The relative changes of turgor-pressure in situ were measured continuously by the hang-weight method [22], which was an improvement upon the method of balance [23]. The leaf turgor-pressure was immediately calibrated by the pressure cham-
The characteristic values of stomata oscillations in adult leaves treated with NaN₃ (10 mM), CCCP (10 μM) separately in G. inflata

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average (mol m⁻² s⁻¹) (the ratio to control)</th>
<th>Amplitude (mol m⁻² s⁻¹) (the ratio to control)</th>
<th>Intensity of oscillation</th>
<th>Period (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.1 ± 0.008 (1.0)</td>
<td>0.08 ± 0.006 (1.0)</td>
<td>0.80</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>NaN₃</td>
<td>0.075 ± 0.006 (0.75)</td>
<td>0.057 ± 0.005 (0.71)</td>
<td>0.76</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>CCCP</td>
<td>0.078 ± 0.006 (0.78)</td>
<td>0.061 ± 0.005 (0.76)</td>
<td>0.78</td>
<td>21 ± 4</td>
</tr>
</tbody>
</table>

* The values were calculated by averaging the data obtained from the five typical cycles that showed the highest amplitude in eight independent measurements. The intensity of the oscillation was defined as the ratio of the amplitude to the average within a cycle.

Table 2
The correlation between the intensity of oscillations and metabolic energy, inhibitors, root proportions or VPD in adult leaves of G. inflata

<table>
<thead>
<tr>
<th>Index</th>
<th>Coefficients of correlation between the intensity of oscillations and the index</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaN₃ concentration</td>
<td>−0.346</td>
</tr>
<tr>
<td>CCCP concentration</td>
<td>−0.283</td>
</tr>
<tr>
<td>Relative metabolic rate</td>
<td>0.329</td>
</tr>
<tr>
<td>Root proportion</td>
<td>0.986ᵇ</td>
</tr>
<tr>
<td>VPD</td>
<td>0.967ᵇ</td>
</tr>
<tr>
<td>Lag of stem flux</td>
<td>0.956ᵇ</td>
</tr>
</tbody>
</table>

*a* The coefficients were calculated from the data in Figs. 1–4 by the function of correlation in the software of Excel.  
*b* *P*<0.01.
treatments with different concentrations of CCCP (Fig. 1 A) or NaN₃ (Fig. 1 B).

3.2. The turgor and K⁺ content during oscillations

Upon an increase of VPD, the turgor of the leaves (Fig. 2A) fluctuated simultaneously with that of the conductance, but K⁺ content of the guard cells did not show such oscillations (Fig. 2B). Then, the K⁺ concentration of the guard cells remained constant during the initial 30 min and declined slowly over the latter time period. This result suggested that the stomatal oscillations were not induced by K⁺ concentration in the guard cells, but resulted from the fluctuation of the leaf turgor-pressure.

3.3. Effect of vapour pressure deficit

VPD calculated from relative humidity and temperature is a comprehensive index that was used as transpiration demand [15,26]. The intensity of stomata oscillations increased in ‘S’-type curve with the increase of VPD in the adult leaves (Fig. 3). The maximum value of the intensity of oscillations upon VPD increase was about 0.8, and the minimum value of VPD required to initiate the oscillations was about 5.8 mPa Pa⁻¹ by analysing the data. The threshold may be changed with different plants, environmental conditions and the sensitivity of the instrument.

3.4. Effect of root and lag of stem flux

When parts of the roots were cut off the average of the leaf conductance increased, but the amplitude of the oscillations of leaf conductance as well as the lag (delayed time compared to transpiration) of the stem flux decreased (Fig. 4A) under high VPD (30 ± 4 mPa Pa⁻¹). The intensity of the oscillation increased linearly with the increase of the proportion of the retained roots from 1/4 to whole root. The consistence of changes between the lag of stem flux and the intensity of the oscillation was used as one of the hydropassive evidence, whereas there was still a small lag of stem flux when the intensity of oscillation could not be detected (Fig. 4B). This means that a minimum of 1/4 of root and lag of stem flux may be required in stomata oscillations in the plant. The intensity of the oscillation was found to be low in old, medium in young and high in adult leaves.

4. Discussion

In this study, data are presented showing the evidence of hydropassive movement in stomata oscillations. The data from the metabolic inhibitor treatments showed that the energy source of stomata oscillations induced by high VPD was not
metabolic dependant (Fig. 1, Table 1). The stomata oscillation may not be dependant on the ATP levels, pH gradients and membrane potentials based on the result that CCCP could not inhibit the oscillations (Fig. 1A) while CCCP could non-specifically reduce the metabolism and ATP levels [5] and short-circuits all pH-gradients and membrane potentials [4]. We could presumed that stomata oscillation may not be ABA inducible because NaN3 could block ABA-induced stomata closure [8] but could not inhibit the oscillations (Fig. 1B). The transportation of K+ across the membrane is a metabolic energy dependant process [5]. The gain and loss of the turgor in stomata oscillations was not be a process metabolic adjustment regulated by the changes in guard cell K+ concentration (Fig. 2B), which was similar to the initial humidity-dependent stomata movements in V. locusta [9].

The oscillations of the leaf turgor-pressure was induced by the non-synchronization between the transpiration demand and provide by the stem flux, which may be the direct cause of the stomata oscillations by analysing the change dynamics of both indexes (Fig. 2). The leaf lost its water by transpiration and obtained water from stem by stem flux, but the lag of the stem flux may cause a water deficit in the leaves sufficient to stimulate a temporary closure of the stomata. The closure of the stomata reduces transpiration, allowing the water provides from the stem to catch up and the stomata to then reopen [19]. In the experiment, there was proportional changes among the lag of stem flux, the retained parts of root and intensity of stomata oscillations Fig. 4. The lag of the stem flux was believed to be induced by the resistance of water transportation in the root [3,24], which may also be the main cause of the oscillations of leaf turgor-pressure. A minimum of 1/4 of roots was required to induce suitable lag of stem flux and the fluctuations of leaf turgor-pressure for stomata oscillations (Fig. 4).

The value of VPD must be high enough (more than a threshold) to induce the stomata oscillations (Fig. 3), which was supported by the results of the RLC model analysis [18] in G. inflata under

![Fig. 2. The turgor pressure of the leaf (A) and the K+ concentration [K+] of the guard cells (B) during conductance oscillations (g) induced by the increase in VPD. The arrow pointed the time of increasing VPD from 10 to above 20 mPa Pa⁻¹ by the method of blowing dry air. The other growing conditions of the plants were described in Section 2. The coefficients of correlation was 0.998 (P < 0.01) between leaf turgor-pressure and leaf conductance.](image-url)
Fig. 3. The effect of VPD on the intensity of stomatal oscillations in the adult leaf of G. inflanta. The values were estimated from the data of the eight duplicates of the diurnal dynamics of leaf conductance and VPD. The amplitudes and the averages, which were used to calculate the intensity of the oscillation, were estimated within each cycle. The corresponding values of VPD were estimated at the middle of the cycle. The fit line is given by the following function ($r^2 = 0.967$): Intensity of stomatal oscillation ($I$) = $(1.2 + 52 \exp ((5.8 - \text{VPD})/3.6))^{-1}$

arid desert conditions. The stomata oscillations were also induced by increases in VPD in the mangrove Avicennia germinans [15]. It was very recently reported that the stomata apertures oscillated at high evaporative demand by the direct observation of stomata and model analysis in Xanthium strumarium L. [26]. The adult and young leaves exhibited stronger intensity of oscillations than the old ones (Fig. 4), which was similar to the observations of the cyclical gain and loss in turgor of the youngest pair of leaves [15]. It means that the stomata oscillations are a type of responses to the severe environment in the plant. The leaves may lose some of the adaptability to the arid environment during their senescence. The mechanism and threshold controlling the hydropassive stomata oscillations are important because these phenomena may be the result of the artful coupling in the plant, which may be used to increase the water use efficiency in agriculture and vegetation recovering in the arid and semiarid regions.

Acknowledgements

We thank Professor J.H. Weil for the gracious direction and revising on the manuscript, Dr. W.H. Shen and Dr. Y.H. Lu for the comments and help. This work was supported by the National Key basic Research Special Funds (NKBRSF No. G1999011705), the national scientific foundation of China (project 39770447) and the research foundation of excellent teachers from the Educational Ministry in China.

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