

# Stomatal movement in response to long distance-communicated signals initiated by heat shock in partial roots of *Commelina communis* L.

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Received April 8, 2005; accepted May 26, 2005

**Abstract** The systematic or long-distance signal transmission plays crucial roles in animal lives. Compared with animals, however, much less is known about the roles of long-distance signal communication in plant lives. Using the model plant *Commelina communis* L., we have probed the root to shoot communication mediated by heat-shock signals. The results showed that a heat shock of 5 min at 40°C in partial roots, i.e. half or even 1/4 root system, could lead to a significant decrease in stomatal conductance. The regulation capability depends on both heat shock temperature and the amount of root system, i.e. with higher temperature and more roots stressed, the leaf conductance would decrease more significantly. Interestingly, the stomatal regulation by heat shock signal is in a manner of oscillation: when stomata conductance decreased to the lowest level within about 30 min, it would increase rapidly and sometimes even exceed the initial level, and after several cycles the stomata conductance would be finally stabilized at a lower level. Feeding xylem sap collected from heat-shocked plants could lead to a decrease in stomata conductance, suggesting that the heat shock-initiated signal is basically a positive signal. Further studies showed that heat shock was not able to affect ABA content in xylem sap, and also, not able to lead to a decrease in leaf water status, which suggested that the stomatal regulation was neither mediated by ABA nor by a hydraulic signal. Heat shock could lead to an increase in xylem sap H<sub>2</sub>O<sub>2</sub> content, and moreover, the removal of H<sub>2</sub>O<sub>2</sub> by catalase could partially recover the stomatal inhibition by xylem sap collected from heat-shocked plants, suggesting that H<sub>2</sub>O<sub>2</sub> might be able to act as one of the root signals to control the stomatal movement. Due to the fact that heat-shock and drought are usually two concomitant stresses, the stomatal regulation by heat-shock signal should be of significance for plant response to stresses. The observation for the stomatal regulation in an oscillation manner by presently identified new signals should contribute to further understanding of the mystery for the plant systematic signaling in response to stresses.

**Keywords:** root signal, leaf conductance, heat shock, long-distance, signal transmission, abscisic acid.

It is well known that animals are able to positively respond to environmental stimuli, thereby avoiding or reducing the possible injuries or impacts on them, and the base for the rapid and positive responses is long-distance signal transmission mediated by the nerve system. Like animals, some sensitive plants or organs are also of the capability to make rapid and positive responses to environmental stimuli. For normal plants, which are not able to move in their lifetime, it is interesting to know whether they are able to positively respond to environmental stimuli through the signal transmission. In recent years, extensive investigations have been carried out to probe cellular signal transduction, and it has been well established that plant cells are of the capabilities to positively respond to environmental stimuli<sup>[1–7]</sup>. Advanced plants are multi-cell organisms whose life activities are dependent on the coordination and interaction among cells. Although investigations on cellular stress signaling have provided important information on plant responses to environmental stimuli, much less is known about the systematic responses of the whole plant based on a long-distance signal transmission.

During the past years, many studies have been carried out to probe the root to shoot communication under water deficit condition<sup>[8–13]</sup>, and it has been suggested that abscisic acid (ABA) might be able to act as a root to shoot signal to regulate stomatal movement and leaf growth. In recent years, however, with investigations expanding further, it has been suggested that ABA might not be a universal signal, due to the fact that in many cases an increase in xylem ABA content could not lead to a reduction in leaf conductance<sup>[8,13–16]</sup>. ABA distribution is believed to be controlled by pH, and hence it has been proposed that pH might be able to act as an ABA-coordinating signal to regulate leaf conductance<sup>[9–11]</sup>. The studies on ABA and pH signals have provided strong evidence that plants are able to positively respond to environmental stimuli through the long-distance signal communication.

The key point for systematic responses is “local-sense” and “whole-response”, which means that signal transmission should be triggered in an earlier stage of stress when no serious injuries or impacts occurred on

the plant physiology. Thus the systematic response mediated by the long-distance signal transmission is more rapid and active, or in other words, more similar to the signal transmission mediated by the nerve system in animals. While extensive investigations have been carried out on the root to shoot communication under water deficit condition, lots of questions are still not fully addressed, e.g. besides ABA and pH, it is not clear whether there are some other signals, which are able to mediate the systematic responses, and moreover, it is not clear how the positive signals can be transmitted from root to shoot against a downstream direction of the water potential from shoot to root. Understandably, it is of high significance to further reveal the mechanisms for plants to actively respond to environmental stimuli based on a long-distance signal transmission.

In field conditions, every kind of environmental stress is usually mingled with each other, and drought and heat stress is just such a situation. In some area, while soil temperature in lower part is still lower, the hot sunshine may lead to a heat shock with a temperature over 40°C in the surface part of the soil. Here, we report that a heat shock in partial root system may trigger a long-distance signal transmission, mediated neither by ABA nor pH, thereby regulating stomatal movement. The present investigation has provided further information on long-distance signal transmission and therefore is of significance for further revealing the profound mystery of the “local-sense” and “whole-response”.

## 1 Materials and methods

### 1.1 Plant material

Seeds of *Commelina communis* L. were germinated in wet sand, when the first pair of leaves appeared, the plants were transplanted to 90 mm×150 mm pots containing flower nutrition soil and sand mixed with a ratio of 1:1. Plants were grown under a condition of day/night temperature 28–26°C and light intensity 300  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , and 3–4 weeks later they were used to do the experiment. In the root-split experiment, after the first pair of leaves appeared, the root system were equally divided into two parts and transplanted to

two pots coupled with each other, and 3–4 weeks later the plants were used to do experiments. In the experiment aimed to collect xylem sap, when the first pair of leaves appeared, the plants were transplanted to specially designed 60 mm×200 mm pots with a dimension just compatible to a pressure chamber, and 3–4 weeks later the plants were used to collect xylem.

### 1.2 Heat shock and leaf conductance measurement

In a preliminary experiment it was found that the heat-shock triggered signal transmission was very fast, to make a heat-shock treatment rapidly and quantitatively an incubator has been adopted. Partial roots were first treated at different temperature for a specific time, then leaf conductance was immediately measured using a prometer (LI 1600, Li-Cor Inc, Lincoln, NE, USA). Due to the big differences in leaf conductance among different leaves, to ensure the reliability of the measurement, for each treatment, four upper fully expanded leaves in every four individual plants were measured. In experiments aimed to collect xylem sap, after treatment for 10 min at 40°C, the root system was sealed in a pressure chamber and xylem saps were collected at a transpiration rate.

### 1.3 Leaf water potential, osmolarity determination and xylem sap collection

Leaf water potential was determined using a pressure chamber<sup>[6]</sup>. To determine the leaf water potential, the whole leaf was cut off with a razor blade at the sheath base, then immediately sealed in a pressure chamber with the leaf blade left outside. A pressure was applied slowly until a small drop of sap just began to appear, and the pressure applied was just the negative value of the leaf water potential. Xylem sap osmolarity was determined using a vapor pressure osmometer (Model 5500, Wewscor, INC, Logan, UT, USA). To collect xylem sap, plants were cut at stem base, then immediately sealed in a pressure chamber. A pressure was applied until saps came out at a rate equivalent to the transpiration rate, and 100 µL of sap was collected from each plant with the first drop of sap discarded.

### 1.4 ABA determination

ABA analyses were carried out using the radioimmunoassay (RIA) method as described by Quarrie *et al.*<sup>[17]</sup>. The highly specific monoclonal antibody (Mac 252) was provided by Dr. Quarrie S. A. (John Innes Centre, UK). 50 µL of crude extracts was mixed with 200 µL phosphate-buffered saline (pH=6.0), 100 µL diluted antibody solution and 100 µL <sup>3</sup>H-ABA (about 8000 cpm) solution. The reaction mixture was incubated at 4°C for 45 min and the bound radioactivity was measured in 50% saturated (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-precipitated pellets with a liquid scintillation counter.

### 1.5 H<sub>2</sub>O<sub>2</sub> and pH determination

H<sub>2</sub>O<sub>2</sub> was determined according to the method of Bellincampi *et al.*<sup>[18]</sup>. Briefly, 500 µL xylem sap was mixed with 500 µL reaction buffer. The reaction buffer consisted of 500 µmol/L ferrous ammonium sulfate, 50 mmol/L H<sub>2</sub>SO<sub>4</sub>, 200 µmol/L xylenol orange and 200 mmol/L sorbitol. After incubating for 1 h, the absorbance at 560 nm was measured and H<sub>2</sub>O<sub>2</sub> content was determined according to a standard curve. pH was determined using a pH meter (ORION-PH meter, Orme Scientific, Manchester, UK).

### 1.6 Feeding experiment

The first pair of fully expanded leaves was used in all feeding experiments. Whole leaf was cut in distilled water, and then immediately put into a 1.5 mL vial with the sheath immersed into equilibrating solution (1 mmol/L CaCl<sub>2</sub>, 1 mmol/L KH<sub>2</sub>PO<sub>4</sub>, 1 mmol/L K<sub>2</sub>HPO<sub>4</sub>, 0.1 mmol/L MnSO<sub>4</sub> and 0.1 mmol/L MgSO<sub>4</sub>). Leaves were firstly allowed to equilibrate under the following condition: light intensity, 250 µmol·m<sup>-2</sup>·s<sup>-1</sup>; temperature, 25°C; and humidity, 65%, after that, the leaves were transferred into different kinds of feeding solutions and the conductance was monitored at different times with the time 0 as a reference. In experiments in which H<sub>2</sub>O<sub>2</sub> was fed, leaf conductance was measured 1 h after H<sub>2</sub>O<sub>2</sub> started to be fed. For each treatment, six leaves were fed.

## 2 Results

Leaf conductance was very sensitive to heat shock

signal, and a heat shock treatment in partial root could lead to a significant decrease in leaf conductance. Fig. 1 shows, when a heat shock in 1/2 root system was carried out for 5 min at 55°C, a 90% decrease in the leaf conductance could be observed within 30 min. Interestingly, leaf conductance responded to heat shock in a manner of oscillation, i.e. when leaf conductance decreased to the lowest level it would rapidly increase again, and after several cycles the leaf conductance would finally sustain at a relatively low level. The degree of the decrease in leaf conductance depended on heat shock temperature and the amount of root, but the regulation manner was basically the same for different temperature or different amount of root. A heat shock at 40°C was able to significantly reduce the leaf conductance (Fig. 1), and furthermore, a heat shock at a temperature as low as 35°C was also able to lead to a decrease in the leaf conductance (data not shown).

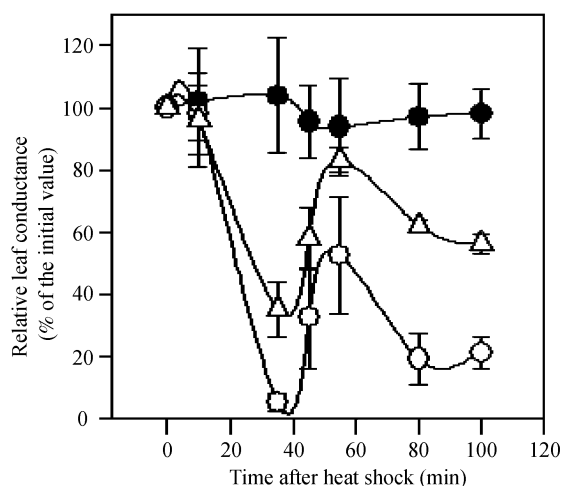


Fig. 1. The effect of heat shock on leaf conductance. ●, No heat shock; ○, heat shock at 55°C for 5 min; △, heat shock at 40°C for 5 min.

Theoretically, the decrease in leaf conductance might be caused by two different mechanisms: one is the so-called positive signal, which contributes to close stomata and is activated under stress condition; and another is the so-called negative signal, which contributes to open stomata but is de-activated under the stress condition. To see whether the characteristic of the heat shock signal is positive or negative, further experiments were performed, in which 1/2 non-stressed root system was removed in order to mimic

the heat shock-induced production of negative signals, and the results indicated that the removal of 1/2 root system had no effects on leaf conductance, suggesting the heat shock signal is not a negative signal. Further results showed that the removal of the stressed root system was able to partially recover the leaf conductance, indicating that the heat shock signal is a positive signal (Fig. 2).

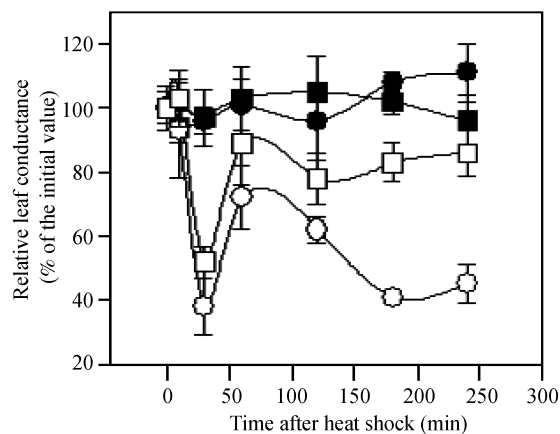


Fig. 2. Recovery of leaf conductance after relief of heat shock. ●, No heat shock; ■, removal of 1/2 roots; ○, heat shock 1/2 roots at 40°C for 5 min; □, removal of the roots treated by heat shock.

Abscisic acid (ABA) and pH are well-established signals regulating stomatal movement under water deficit condition. To see whether the two signals might be involved in heat shock-triggered signal transmission, the changes in xylem ABA and pH were analyzed, but unfortunately, no changes were found either in ABA content or in pH value (Fig. 3). While heat shock had no effects on xylem ABA content or pH value, it was able to cause a decrease in osmolarity of the xylem sap, which means that heat shock might be able to increase membrane permeability therefore increasing the ion concentrations in xylem sap.

Besides ABA and pH, hydraulic signal is generally thought to be the most important signal to regulate stomatal movement. Due to the fact that heat shock might be able to change the membrane permeability therefore change the root water-conductance, it is reasonable to think that heat shock might be able to trigger a hydraulic signal. As expectedly, heat shock could indeed lead to a change in leaf water potential, but it is an increase but not a decrease that was observed (Fig.

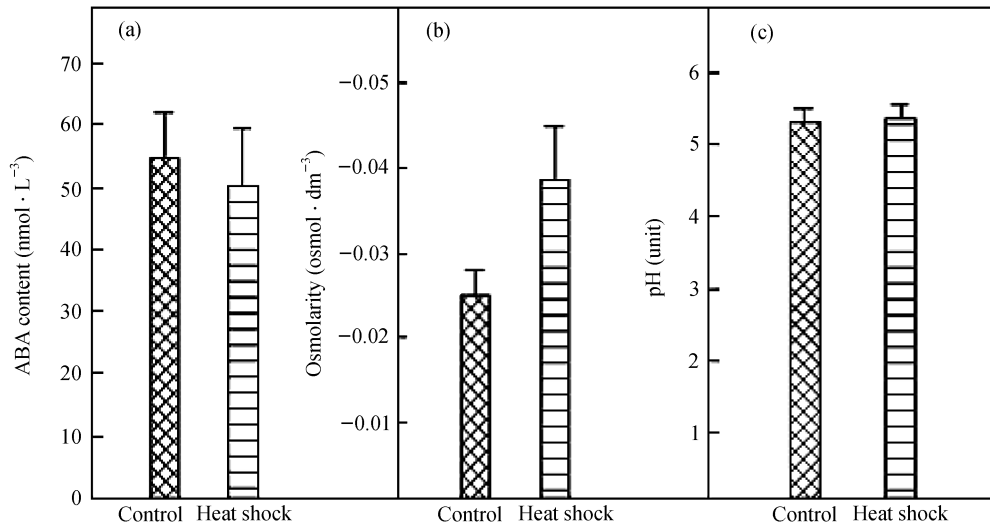


Fig. 3. The effect of heat shock on xylem sap ABA content (a), osmolarity (b), and pH (c).

4). Obviously, the heat shock-induced reduction in leaf conductance was not mediated by a hydraulic signal.

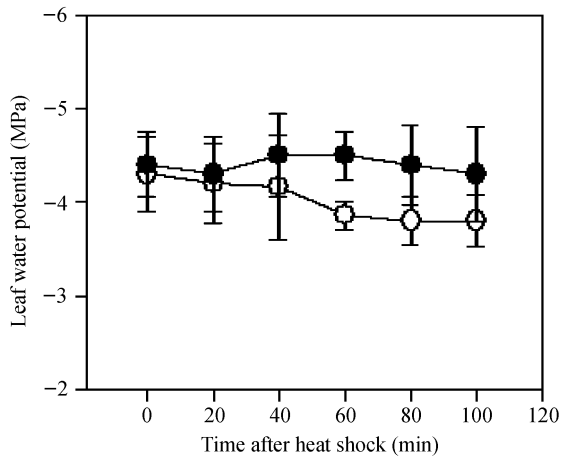


Fig. 4. The effect of heat shock on leaf water potential. ●, No heat shock; ○, heat shock for 1/2 roots at 40°C for 5 min.

It has been increasingly suggested that H<sub>2</sub>O<sub>2</sub> is able to act as a signal to regulate stomatal movement. To see whether H<sub>2</sub>O<sub>2</sub> might be able to act as a heat shock-triggered signal, the changes in xylem sap H<sub>2</sub>O<sub>2</sub> were investigated. The results showed that heat shock could lead to an increase in xylem sap H<sub>2</sub>O<sub>2</sub> content although the time-course for the changes in H<sub>2</sub>O<sub>2</sub> content was not totally identical with the stomatal movement (Fig. 5). Feeding H<sub>2</sub>O<sub>2</sub> could lead to a reduction in the leaf conductance in a concentration-dependent manner (Fig. 6). Furthermore, feeding xylem sap col-

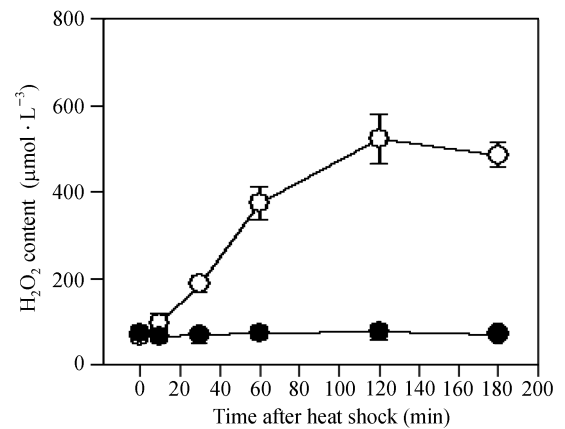


Fig. 5. The effect of heat shock on H<sub>2</sub>O<sub>2</sub> content in xylem sap. ●, No heat shock; ○, heat shock for 1/2 roots at 40°C for 5 min.

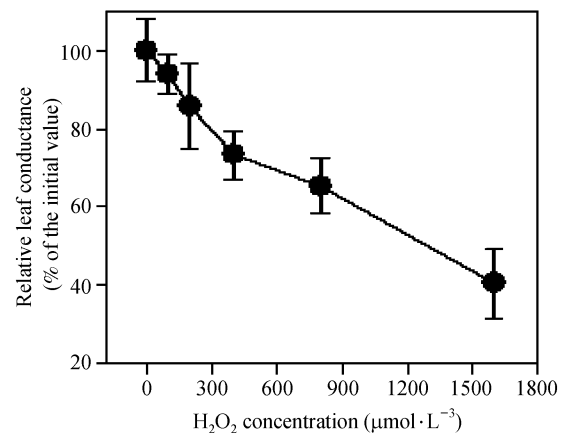


Fig. 6. The effect of feeding H<sub>2</sub>O<sub>2</sub> on leaf conductance.

lected from heat shock-treated plants could also lead to a reduction in the leaf conductance, and importantly, the removal of  $H_2O_2$  with catalase could partially relieve the inhibiting effect on leaf conduction by the xylem sap collected from heat shock treated plants (Fig. 7). These results strongly suggest  $H_2O_2$  might be able to act as a heat shock-triggered signal to regulate stomatal movement through a long-distance signal transmission.

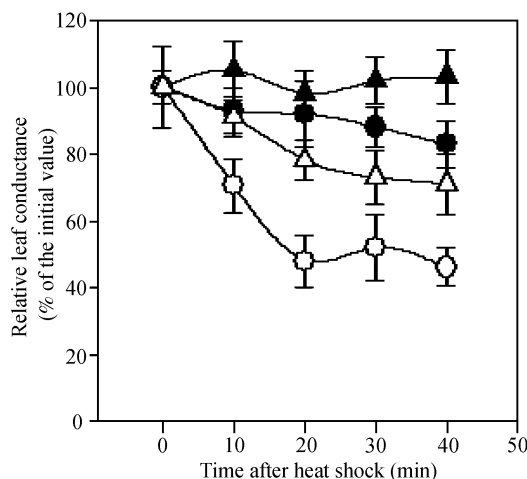


Fig. 7. The effect of feeding xylem sap on leaf conductance. ▲, Feeding artificial xylem sap; ●, feeding xylem sap collected from plants without heat shock; ○, feeding xylem sap collected from plants treated at 40°C for 10 min; △, feeding xylem sap collected from plants treated by heat shock and  $H_2O_2$  was removed by catalase.

### 3 Discussion

The observation of stress signals is a big achievement in the researches on plant stress physiology. ABA is generally thought to be one of the most important signals. But with the researches expanding further, it has been realized that ABA is not a universal signal, alternatively, besides ABA there should be other signals, which are able to mediate systematic responses to environmental stimuli. Some recent studies suggested that pH might be able to act as a signal, independently or coordinately with ABA, to regulate stomatal movement and leaf growth<sup>[9–11]</sup>. In view of the importance of ABA and pH signal, the present study tried to see whether they were able to mediate the signal transmission triggered by heat shock, unfortunately, both of them were not observed to be involved in the signal transmission triggered by heat shock. Although

ABA and pH signals have been paid close attention, it should be noted that neither ABA nor pH signal is more powerful than heat shock signal as to the regulation of stomatal movement. Under drought condition, the inhibition of leaf conductance by ABA or pH signal is normally less than 40%<sup>[8,11]</sup>, but the heat shock especially at a higher temperature might be able to cause a complete stomatal closure, and even for a weaker heat shock (e.g. below 40°C or less than 1/4 root system) the leaf conductance might be significantly reduced. The key point for long-distance signal transmission is to realize “local sense” and “systematic response”, which aims to make the adaptive responses before plants are seriously injured. Drought and heat shock are usually mingled with each other. Just like drought, heat shock is also a kind of localized stress, e.g. while the soil temperature in lower part is still lower, hot sunshine may lead to a heat shock with a temperature over 40°C in upper part of the soil. Understandably, the sensitive regulation of stomatal movement by the heat signal is of high significance for plants to resist or adapt to environmental stresses.

Many studies suggest that cold stress might lead to a decrease in root water-conductance<sup>[19–22]</sup>, so it appears that heat shock might lead to an increase in the water-conductance therefore lead to an increase in leaf water potential. As expected, an increase in leaf water potential was observed, from which it can be concluded that the heat shock-induced reduction in leaf conductance was not mediated by the hydraulic signal.  $H_2O_2$  is believed to be an important signal<sup>[23–26]</sup>. It is well known that heat shock might lead to a production of  $H_2O_2$ <sup>[27–29]</sup>, so it is interesting to know whether  $H_2O_2$  might be able to act as a heat shock-triggered signal. The results, here, strongly suggested that  $H_2O_2$  might be able to mediate the root to shoot communication. Nevertheless, it should be noted that the root to shoot communication triggered by environmental stimuli is rather complicated, e.g. the root signal triggered by drought has been increasingly proved to be a complex signal but not a mono-signal as believed before<sup>[30,31]</sup>. Just like that in the case of drought, the root to shoot communication triggered by heat shock might be also a complex signal since the removal of  $H_2O_2$  could only partially but not completely relieve the in-

hibition of leaf conductance. As mentioned above, heat shock might likely lead to changes in concentration and composition of ions in xylem sap.  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  are well-known ions playing important roles in stomatal movement, so whether the changes in concentration and composition of the ions in xylem sap are possibly involved in the root to shoot communication deserves to attract more attentions. Furthermore, it has been proved that heat shock could trigger physical signals such as electronic wave transmission<sup>[32–34]</sup>, so besides ions whether some physical signals might be involved in the signal transmission also deserves to be further investigated.

It is well known that *Commelina communis* L. is a model plant for researches on stomatal movement. The present study has, for the first time, observed that heat shock can trigger a long-distance signal transmission in *Commelina communis* L. It is important to know whether heat shock might be able to trigger such a signal transmission in other plant species especially crop plants. We have once performed some preliminary experiments using sunflower and maize plants, and also observed similar effects of heat shock on stomatal movement although these plants were less sensitive to heat shock compared with *Commelina communis* L. So, it appears that the long-distance signal transmission triggered by heat shock could universally occur in different plant species. Advanced plants are multi-cell organisms with life activities dependent on synergetic coordination and regulation among cells. The present study has provided important information about how plants are able to rapidly and positively respond to environmental stimuli by systematic signal communication, and it should be of significance both for the expansion of plant science and agricultural production.

**Acknowledgements** This work was supported by the National Basic Research Program of China (Grant No. 2003CB114300) and National Natural Science Foundation of China (Grant Nos. 30270135 & 30470160).

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