EVALUATION OF STOMATAL ACTIVITY BY MEASURING LEAF TEMPERATURE DYNAMICS

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EVALUATION OF STOMATAL ACTIVITY
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KOUTAKI M., EGUCHI H. and MATSUI T. Evaluation of stomatal activity by measuring leaf temperature dynamics. BIOTRONICS 12, 29-42, 1983. For evaluation of stomatal activity, dynamic characteristics of leaf temperatures in cucumber plants were analyzed under radiation of tungsten light in a controlled air of 30°C and 40% relative humidity. When leaves were radiated, leaf temperature rose and appeared in oscillation. The oscillation of leaf temperature was caused by stomatal movement, and the degree of stomatal movement was evaluated by summing the amplitudes of the leaf temperature oscillation. Nodal distribution of the leaf temperatures was found clearly in a plant, and it appeared conceivable that the stomatal movements in the older and the younger (not fully expanded) leaves were slacker than those in the other leaves. The stomatal movement in the older leaves was able to be promoted when the stomatal movement in the leaves other than the older leaves was inhibited or the other leaves were excised. The stomatal movement in the younger leaves, however, was still slack and not promoted even in the same treatments where the stomatal movement in the leaves other than the younger leaves was inhibited or the other leaves were excised. These facts suggest that the older leaves maintain stomatal activity enough, but are sluggish in stomatal movement while stomata in the other leaves are acting sufficiently, and also suggest that the functions of stomata in the younger leaves have not yet been developed, and the stomatal activity is lower at all times. Thus, the stomatal activity was evaluated by leaf temperature analysis in the on-line system.

INTRODUCTION

Stomatal movement is one of the important functions in the leaf (4, 5, 27, 28, 31). So, the analysis of the stomatal movement could give useful information about the leaf responding to the environment. However there is difficulty in measuring stomatal movement directly in the intact leaf exposed to the environment. On the other hand, it is known that stomatal movement is responsible for leaf temperature, as well as environmental factors (9, 10, 21): Matsui and Eguchi (19, 20) reported the effects of air temperature, humidity, light and wind velocity on leaf temperature. Furthermore, Shiraishi et al. (29) and Hashimoto et al. (13) analyzed the relation between leaf temperature and stomatal aperture observed under the scanning electron microscope by using detached leaves. From these studies, it appears
conceivable that the stomatal movement can be examined through the analyses of relation between leaf temperature and environmental factors and can be used as an index for evaluation of leaf activity.

The present paper deals with analysis of dynamic characteristics of leaf temperature for evaluation of stomatal activity in on-line measurements.

MATERIAL AND METHODS

Cucumber plants (Cucumis sativus L. var. Hort. Chojitsu-Ochiai No. 2) were used in the experiments. The plants were potted in moistened Vermiculite and grown at an air temperature of 23±1°C and a relative humidity of 70±5% in a phytotron glass room. The intact leaves of respective plants at 5 leaves stage (20 days old plant) and at 10 leaves stage (30 days old plant) were used as specimens. The copper-constantan thermocouple (Ø 0.1 mm) was inserted into the leaf (25), and the voltage (0–2 mV) of the thermocouple was recorded as the temperature (0–50°C) in course of time (chart speed of 1 cm min⁻¹). In this instrumentation system, the leaf temperature was measured with an accuracy of 0.1°C and with a time constant of about 1 sec at an air temperature of 30±0.5°C, a relative humidity of 40±5% and an air velocity of 0.3±0.1 m sec⁻¹ in a growth chamber. For radiation to leaves, tungsten lamps (7 lamps of 500 W) were used as a light source: The total radiant flux density \( R \) was 176 mW cm⁻² in 0.35–60 μm, which consisted of short-wave radiant flux density \( R_s \) of 52 mW cm⁻² in 0.35–3 μm (4.2±0.6 mW cm⁻² in 0.4–0.7 μm) and longwave radiant flux density \( R_l-R_s \) of 124 mW cm⁻² in 3–60 μm. After keeping the plant in darkness for 12 hr, the leaves were radiated vertically with the tungsten light.

RESULTS AND DISCUSSION

Distribution of temperatures in a leaf

Temperature distribution in a leaf has been observed in several species (9, 12, 13, 24). In this experiment, to examine the temperature distribution in a cucumber leaf, leaf temperatures were measured at 6 points in a leaf as shown in Fig. 1. Figure 2 shows time course pattern of the leaf temperatures at respective measuring points (A–F in Fig. 1) in the 3rd leaf of the cucumber plant at 5 leaves stage. In darkness, all leaf temperatures were kept almost constant at 28±0.5°C. When the leaf was radiated, the leaf temperatures rose rapidly and thereafter appeared in oscillation. This transient pattern of the leaf temperature could be found as a step response to lighting. The pattern of this oscillation varies with air conditions, as reported by Kitano et al. (17); they used air velocity of 0.7 m sec⁻¹ at different air temperatures and humidities. In this experiment carried out under the constant air condition (30°C, 40% RH and an air velocity of 0.3 m sec⁻¹), the leaf temperature oscillation settled in course of time within 2 hr after radiation.

Distinct differences among the leaf temperatures were found in amplitude of oscillation: The amplitudes were higher at E and F points than those at other points. From these patterns, it could be conceivable that the stomatal movement is more
active at the part closer to the leaf edge, as compared with that at central midrib. Thus, E point was selected for the measurement to represent the leaf temperature.

**Relationship between leaf temperature and stomatal movement**

The oscillation of the leaf temperature in radiation can be considered to be
caused by stomatal movement (1–3, 11, 13–15, 29). So, the relation between leaf temperature and stomatal movement was examined on the basis of heat balance (8, 9, 16, 24, 26) of a leaf. Figure 3 shows the schematic diagram of heat balance of the leaf. From heat balance of the leaf, it could be conceivable that the leaf temperature oscillation is caused by change in the latent heat through stomatal movement. The metabolic heat was remarkably small and was neglected. On the basis of heat balance of the leaf, transpiration rate and leaf conductance were calculated from following equations (17),

\[
E = \frac{6 \times 10^{-2}}{\lambda} \left\{ R_t - (1 - A_b) R_s - 2 \sigma 273.16 + T_i^4 \right\} - 10^8 s m \frac{dT_i}{dt} - \frac{2 \times 10^8 C_p \rho (T_i - T_a)}{r_{ah}} \right\} \tag{1}
\]

and

\[
C_l = \left[ \frac{1.2 \times 10^{-4} \left\{ W(T_i) - \frac{R_H}{100} W(T_a) \right\}}{E - r_{ah} L e^{2/3}} \right]^{-1} \tag{2}
\]

where: \(E\), transpiration rate (g cm\(^{-2}\) min\(^{-1}\)); \(R_t\), total (0.35–60 \(\mu\)m) radiant flux density (mW cm\(^{-2}\)); \(R_s\), shortwave (0.35–3 \(\mu\)m) radiant flux density (mW cm\(^{-2}\)); \(A_b\), coefficient of shortwave absorption by leaf; \(\varepsilon\), emissivity of leaf; \(\sigma\), Stefan-Boltzmann constant (mW cm\(^{-2}\) K\(^{-4}\)); \(T_i\), leaf temperature (°C); \(T_a\), air temperature (°C); \(s\), specific heat of leaf (J g\(^{-1}\) °C\(^{-1}\)); \(m\), leaf weight.
per unit area (g cm⁻²); \( t \), time (sec); \( C_p \), specific heat of air (J g⁻¹ °C⁻¹); \( \rho \), density of air (g cm⁻³); \( \lambda \), latent heat for vapourization of water (J g⁻¹); \( r_{ah} \), boundary layer resistance of leaf to heat transfer (sec cm⁻¹); \( C_l \), leaf conductance to vapour transfer (cm sec⁻¹); \( W(T_l) \), saturation vapour density at leaf temperature of \( T_l \) (g m⁻³); \( W(T_a) \), saturation vapour density at air temperature of \( T_a \) (g m⁻³); \( RH \), relative humidity of air (%) and \( Le \), Lewis number.

In this experiment, \( A_b \) was 0.26 and \( r_{ah} \) was 0.67 sec cm⁻¹, where measurements of \( A_b \) and \( r_{ah} \) followed the manners reported by Kitano et al. (17). Figure 4 shows leaf temperature, measured transpiration rate, calculated transpiration rate and calculated leaf conductance in the 3rd leaf in a plant at 5 leaves stage, where the other leaves were excised. The transpiration rate was measured by weighing the plant and pot in which the soil surface was covered with polyethylene film to prevent the evaporation. The measured transpiration rate rose rapidly after radiation and appeared in oscillation. In these patterns, the transpiration rate was the maximum (the peak) at the same time when the leaf temperature was the minimum (the dip). The calculated transpiration rate conformed well to the measured values. There was high correlation \((r=0.906, p<0.001)\) between them. Furthermore, the calculated leaf conductance appeared in similar pattern of the measured and calculated transpiration rates. Thus, the pattern of the leaf temperature corresponded to the stomatal movement in their characteristics of the oscillations. On the other hand,

![Graph showing calculated transpiration rate, calculated leaf conductance, measured transpiration rate (●) and leaf temperature in the 3rd leaf (at 5 leaves stage) treated with 10⁻⁴ M ABA and radiated by tungsten light under a constant air condition (30°C, 40% RH).](image)

**Fig. 5.** Calculated transpiration rate, calculated leaf conductance, measured transpiration rate (●) and leaf temperature in the 3rd leaf (at 5 leaves stage) treated with 10⁻⁴ M ABA and radiated by tungsten light under a constant air condition (30°C, 40% RH).
when the stomatal movement was inhibited with abscisic acid (10^{-4} \text{ M} \text{ ABA}) (6, 7, 18, 22, 23, 30), the oscillations were not found in any of leaf temperature, measured and calculated transpiration rates and calculated leaf conductance, as shown in Fig. 5. From these facts, it could be estimated that the pattern of leaf temperature can be used as information about stomatal movement, and it is made possible to evaluate the stomatal activity on the basis of heat balance of the leaf, by measuring the leaf temperature under a constant environmental condition.

**Nodal distribution of leaf temperatures in a plant**

To analyze the effect of aging on stomatal movement, the leaf temperatures were measured in leaves at different ages (at different nodes). Figure 6 shows respective leaf temperatures in a plant at 5 leaves stage. In the older (the 1st) and in the younger (the 5th) leaves, the temperature oscillations were smaller than those in other leaves; the temperature oscillation in the 3rd leaf was the largest. These characteristics were clear in the amplitudes of the leaf temperature oscillations. So, the amplitudes of each temperature oscillation were summed and compared with each other as shown in Fig. 7: The summed amplitudes were the highest in the 3rd

![Fig. 6. Nodal distribution of leaf temperatures in a plant at 5 leaves stage in course of time after radiation of tungsten light under a constant air condition (30\textdegree C, 40\% RH); 1–5 are the respective temperatures of the 1st–the 5th leaves.](image)

![Fig. 7. Leaf length (□) and sum of amplitudes (□) of leaf temperature oscillation in each of the 1st–the 5th leaves in the plant at 5 leaves stage, where the leaf temperatures were measured under a constant air condition (30\textdegree C, 40\% RH) in radiation of tungsten light.](image)
Fig. 8. Nodal distribution of leaf temperatures in a plant at 10 leaves stage in course of time after radiation of tungsten light under a constant air condition (30°C, 40% RH): (a), temperatures of the 1st–the 5th (1–5) leaves; (b), temperature of the 6th–the 10th (6–10) leaves.

Fig. 9. Leaf length (□) and sum of amplitudes (◼) of leaf temperature oscillation in each of the 1st–the 10th leaves in the plant at 10 leaves stage, where the leaf temperatures were measured under a constant air condition (30°C, 40% RH) in radiation of tungsten light.
leaf, but those in the older (the 1st) and the younger (the 5th) leaves were remarkably low. The differences in summed amplitudes between the 3rd leaf and each of the 1st and the 5th leaves were significant at 5% level. These temperature characteristics in leaves at respective ages were also examined in a plant at 10 leaves stage. Figure 8 shows nodal distribution of leaf temperatures in a plant at 10 leaves stage. The temperature oscillations were smaller in the younger (the 9th and the 10th) and in the older (the 1st and the 2nd) leaves, and larger in the 7th and the 8th leaves than those in other leaves. The summed amplitudes and leaf length were illustrated on node number (ages) in Fig. 9. As seen in leaf lengths, the 5th leaf in a 5 leaves stage plant, and the 9th and the 10th leaves in a 10 leaves stage plant were younger and not fully expanded. The 1st leaf was the oldest but small in leaf length. The 8th leaf was fully expanded younger one. The summed amplitudes were the highest in the 8th leaf. On the other hand, in each of the younger and the older leaves, the summed amplitudes were remarkably lower than those in other leaves. The differences in summed amplitudes between the 8th leaf and each of the younger and the older leaves were significant at 5% level. These facts suggest that stomatal move-

Fig. 10. Temperatures of the 1st (1-IIa), the 2nd (2-IIa) and the 8th (8-IIa) leaves in a plant at 10 leaves stage, where $10^{-4}$ M ABA was applied to the 3rd–the 10th leaves (1-I and 2-I are respective temperatures of the 1st and the 2nd leaves in an untreated plant); the leaf temperatures were measured under a constant air condition (30°C, 40% RH) in radiation of tungsten light.

Fig. 11. Temperatures of the 1st (1-IIa), the 2nd (2-IIa) and the 8th (8-IIa) leaves in a plant at 10 leaves stage, where the 3rd–the 10th leaves were covered with aluminium foil to cut off the light (1-I and 2-I are respective temperatures of the 1st and the 2nd leaves in an untreated plant); the leaf temperatures were measured under a constant air condition (30°C, 40% RH) in radiation of tungsten light.
ments in the younger and the older leaves were slacker than those in fully expanded younger leaves.

Estimation of stomatal activity in younger and older leaves

For the stomatal behaviours in the younger and the older leaves, it was presumed that the younger (not fully expanded) and the older leaves would be sluggish in stomatal movements while the stomata are acting sufficiently in the other expanded leaves, and the plants were treated as listed in Table 1.

Figure 10 shows time course patterns of the temperatures of the 1st (1-I) and the 2nd (2-I) leaves in an untreated plant, and of the 1st (1-IIa), the 2nd (2-IIa) and the 8th (8-IIa) leaves in a plant treated in Exp. IIa where the 3rd–the 10th leaves were treated 12 hr before radiation with $10^{-4}$ M ABA. By this treatment, stomatal movement in the 3rd–the 10th leaves was inhibited as observed in the 8th leaf in which the oscillation was not found. In the 1st and the 2nd leaves, the oscillations

![Graph 1](image1)

![Graph 2](image2)

**Fig. 12.** Temperatures of the 1st (1-IVa) and the 2nd (2-IVa) leaves in a plant at 10 leaves stage, where the 3rd–the 10th leaves were excised (1-I and 2-I are respective temperatures of the 1st and the 2nd leaves in an untreated plant); the leaf temperatures were measured under a constant air condition (30°C, 40% RH) in radiation of tungsten light.

**Fig. 13.** Sum of amplitudes of leaf temperature oscillation in each of the 1st and the 2nd leaves in the plant at 10 leaves stage, where the leaf temperatures were measured under a constant air condition (30°C, 40% RH) in radiation of tungsten light in respective treatments: I, untreatment; IIa, applying $10^{-4}$ M ABA to the 3rd–the 10th leaves; IIIa, covering the 3rd–the 10th leaves with aluminium foil; IVa, excising the 3rd–the 10th leaves.
became larger than those in the leaves in the untreated plant. Similar characteristics were also observed from Exp. IIIa where the 3rd–the 10th leaves were kept in darkness by covering those leaves with aluminium foil. Figure 11 shows time course patterns of the temperatures of the 1st (1-I) and the 2nd (2-I) leaves in an untreated plant, and of the 1st (1-IIIa), the 2nd (2-IIIa) and the 8th (8-IIIa) leaves in the plant treated in Exp. IIIa. This treatment resulted in increasing in the amplitudes of leaf temperature oscillation in each of the 1st and the 2nd leaves. In the 3rd–the 10th leaves (covered with aluminium foil), the oscillation did not occur, as found in the 8th leaf temperature. Thus, in the case that only the 1st and the 2nd leaves were radiated while the other leaves were kept in darkness, stomatal movement in the older leaves was promoted. Furthermore, the stomatal activity in the older leaves was examined in a plant treated with excision of the 3rd–the 10th leaves. Figure 12 shows time course patterns of the temperatures of the 1st (1-I) and the 2nd (2-I) leaves in an untreated plant, and of the 1st (1-IVa) and the 2nd (2-IVa) leaves in the plant treated in Exp. IVa. The larger oscillations of leaf temperatures occurred in the 1st and the 2nd leaves in the treated plant, but in the untreated plant, the oscillation was small. Thus, this treatment also resulted in promoting the stomatal movement.

To compare the degree of increase in stomatal movement in the older leaves in three treatments, the respective amplitudes of the leaf temperature oscillations were summed and shown in Fig. 13. The summed amplitudes of the leaf temperature oscillations in the older leaves in treated plants were clearly higher than those in untreated plants; the differences in summed amplitudes between treated and untreated plants were significant at 5% level. This fact suggests that the stomatal movement in the older leaves can be promoted when the stomatal movement in the other leaves is inhibited or the other leaves are excised.

As stated above, the stomatal movement in the younger (not fully expanded) leaves was slacker than that in other leaves. So, the stomatal activity in those leaves in the untreated plant was also lower than that in the other leaves.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Treatments</th>
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<td>Untreatment</td>
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| II          | a), Applying $10^{-4}$ M ABA to the 3rd–the 10th leaves in a plant at 10 leaves stage.  
             | b), Applying $10^{-4}$ M ABA to the 1st–the 8th leaves in a plant at 10 leaves stage. |
| III         | a), Covering the 3rd–the 10th leaves in a plant at 10 leaves stage with aluminium foil to keep the leaves in darkness.  
             | b), Covering the 1st–the 8th leaves in a plant at 10 leaves stage with aluminium foil to keep the leaves in darkness. |
| IV          | a), Excising the 3rd–the 10th leaves in a plant at 10 leaves stage.  
             | b), Excising the 1st–the 8th leaves in a plant at 10 leaves stage. |
| N. B.       | a), treatments for estimating the stomatal activity in older leaves.  
             | b), treatments for estimating the stomatal activity in younger leaves. |
Fig. 14. Temperatures of the 8th (8-IIb), the 9th (9-IIb) and the 10th (10-IIb) leaves in a plant at 10 leaves stage, where $10^{-4}$ M ABA was applied to the 1st–the 8th leaves (9-I and 10-I are respective temperatures of the 9th and the 10th leaves in an untreated plant); the leaf temperatures were measured under a constant air condition (30°C, 40% RH) in radiation of tungsten light.

Fig. 15. Temperatures of the 8th (8-IIIb), the 9th (9-IIIb) and the 10th (10-IIIb) leaves in a plant at 10 leaves stage, where the 1st–the 8th leaves were covered with aluminium foil to cut off the light (9-I and 10-I are respective temperatures of the 9th and the 10th leaves in an untreated plant); the leaf temperatures were measured under a constant air condition (30°C, 40% RH) in radiation of tungsten light.

Younger leaves was estimated by the same treatments (Table 1) as estimated in the older leaves. Figure 14 shows time course patterns of the temperatures of the 9th (9-I) and the 10th (10-I) leaves in an untreated plant, and of the 8th (8-IIb), the 9th (9-IIb) and the 10th (10-IIb) leaves in a plant treated in Exp. IIb where the 1st–the 8th leaves were treated 12 hr before radiation with $10^{-4}$ M ABA. In the 8th leaf treated with ABA, there was no leaf temperature oscillation, and the stomatal movement was inhibited completely. In the 9th and the 10th leaves in the treated plant, the leaf temperatures appeared in smaller oscillation similar to those in the untreated plant. These patterns indicate that the stomatal movement in the younger (the 9th and the 10th) leaves could not be promoted by the treatment. These characteristics were also found in the Exp. IIIb where the 1st–the 8th leaves were covered with aluminium foil to cut off the light. Figure 15 shows time course patterns of the temperatures of the 9th (9-I) and the 10th (10-I) leaves in an untreated plant, and of the 8th (8-IIIb), the 9th (9-IIIb) and the 10th (10-IIIb) leaves in the plant treated in Exp. IIIb. In the 8th leaf kept in darkness, there was no leaf temperature oscillation. Between treated and untreated plants, any appreciable differ-
Fig. 16. Temperatures of the 9th (9-IVb) and the 10th (10-IVb) leaves in a plant at 10 leaves stage, where the 1st–the 8th leaves were excised (9-I and 10-I are respective temperatures of the 9th and the 10th leaves in an untreated plant); the leaf temperatures were measured under a constant air condition (30°C, 40% RH) in radiation of tungsten light.

Fig. 17. Sum of amplitudes of leaf temperature oscillation in each of the 9th and the 10th leaves in the plant at 10 leaves stage, where the leaf temperatures were measured under a constant air condition (30°C, 40% RH) in radiation of tungsten light in respective treatments: I, untreated; IIb, applying $10^{-4}$ M ABA to the 1st–the 8th leaves; IIIb, covering the 1st–the 8th leaves with aluminium foil; IVb, excising the 1st–the 8th leaves.

ences were scarcely found in the leaf temperature oscillations in the 9th and the 10th leaves. Thus, it was impossible to promote stomatal movement in the younger (the 9th and the 10th) leaves in this treatment. Furthermore, the experiment was carried out to estimate the stomatal activity in the younger leaves in a treatment where the 1st–the 8th leaves were excised. Figure 16 shows time course patterns of the temperatures of the 9th (9-I) and the 10th (10-I) leaves in an untreated plant, and of the 9th (9-IVb) and the 10th (10-IVb) leaves in the plant treated in Exp. IVb. These patterns of the leaf temperatures were similar to each other; clear differences were not found in leaf temperature oscillation between treated and untreated plants. Thus, this treatment did not promote the stomatal movement.

Figure 17 shows comparison of summed amplitudes of leaf temperature oscillation in each of the 9th and the 10th leaves in respective treatments. There were not any appreciable differences in summed amplitudes between treated and untreated plants. Thus, in the younger (not fully expanded) leaves, stomatal movement was still slack even in the respective treatments. This fact suggests that the stomatal movement can not be promoted because the functions of stomata have not yet been developed enough in these younger leaves.
Conclusion

In nodal distribution of leaf temperatures, it was found that the stomatal movement was the most active in the 3rd leaf from shoot apex, and was slacker in the younger (not fully expanded) leaves and in the older leaves. In an attempt to estimate the stomatal activities in the younger and the older leaves, the stomatal movement was increased only in the older leaves but not in the younger leaves. From this fact, it could be conceivable that the older leaves are sluggish in stomatal movement in spite of maintaining the stomatal activity enough while the stomata in the other leaves are acting sufficiently, and could be also conceivable that the functions of stomata in the younger (not fully expanded) leaves have not yet been developed and the stomatal activity is lower out of relation to the physiological condition of the other leaves in a plant. Thus, this instrumentation made it possible in the on-line system to obtain the information about stomatal activity.

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