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AN IMPROVED CUVETTE FOR INDIVIDUAL EVALUATIONS OF GAS EXCHANGE PARAMETERS ON ADAXIAL AND ABAXIAL LEAF SURFACES UNDER DIFFERENT AIR CURRENTS

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YASUTAKE D., KITANO M., HAMAKOGA M., ARAKI T., NAGASUGA K. and SUZUKI Y. *An improved cuvette for individual evaluations of gas exchange parameters on adaxial and abaxial leaf surfaces under different air currents.* BIOTRONICS **29**, 33–42, 2000. For individual evaluations of gas exchange parameters on adaxial and abaxial leaf surfaces, the Parkinson leaf cuvette (PP Systems. Ltd., UK) was improved by providing individual air paths to the respective upper (adaxial) and lower (abaxial) leaf cuvettes, where air current in each cuvette was also adjustable independently. The performance of the improved cuvette system was examined by using a wetted filter paper and a cucumber leaf. The three different modes of measurement, i.e. the upper cuvette mode, the lower cuvette mode and the both cuvettes mode, were selected successively, and leaf boundary layer conductance on each surface was arbitrarily adjusted in the range of 0.3 mol $m^{-2} s^{-1}$ to 1.0 mol $m^{-2} s^{-1}$. The heterogeneity between adaxial and abaxial surfaces of the cucumber leaf was found in gas exchange parameters such as stomatal conductance and rates of transpiration and photosynthesis, and further stomatal response to air current was revealed quantitatively by change in stomatal conductance.

Key words: leaf cuvette, adaxial and abaxial surfaces, stomatal response to air current, heterogeneity.

INTRODUCTION

Various types of the system for measuring leaf gas exchange have been developed and widely used for evaluations of photosynthesis, transpiration and stomatal function of a leaf (e.g. *2, 3, 7*). In those systems, gas exchange parameters such as stomatal conductance are evaluated without distinction between adaxial and abaxial leaf surfaces, on the assumption of the homogeneity between the both surfaces. However, the homogeneity between adaxial and abaxial surfaces is not the case because of differences in stomatal frequency, epidermal system, mesophyll structure and air current (e.g. *6*), and difference in CO_2 gas concentration between both leaf surfaces has been detected and related to relative contribution of photosynthesis of each surface (*5, 10*). Therefore, it is desired to establish the system for quantitative and individual evaluations of gas exchange parameters on adaxial and abaxial leaf surfaces. Such system might be also applicable to studies on scaling-up of gas exchange parameters over heterogeneous surfaces.

Furthermore, most of the studies on stomatal responses to environmental factors have been focused on the responses to light and humidity, and little information on stomatal response to air current has been obtained (*6*). Effects of air current on leaf photosynthesis and root water absorption have been studied by using a wind tunnel (*9, 11*), and further stomatal oscillation has been observed in a cucumber plant beeing exposed to rapid change in air current in a wind tunnel (*4*). However, leaf cuvettes widely used have not been applicable to analysis of stomatal response to air current, because the condition of air current in those leaf cuvettes is fixed so high that leaf boundary layer resistance is not a limiting factor for the leaf gas exchange. In the present study, the Parkinson leaf cuvette (PLC; PP Systems. Ltd., UK) was improved for individual evaluations of gas exchange parameters and independent control of air current on adaxial and abaxial leaf surfaces.

SYSTEM

For individual evaluations of gas exchange parameters on adaxial and abaxial leaf surfaces under different air currents, we improved the Parkinson leaf cuvette (PLC), which has been widely used for measuring leaf gas exchange. Figure 1 shows a schematic diagram of the system improved. The system consists of the improved PLC, air paths, a gas analyzing unit (CIRAS-1; PP Systems Ltd., UK) and newly appended devices such as flow meters, solenoid valves and potentiometers. CIRAS-1 is equipped with CO_2 and H_2O infrared gas analyzers, mass flow controllers and air pumps *etc*., which can be operated through a computer. This system for measuring leaf gas exchange is the type of open system. That is, the ambient air is pumped into CIRAS-1 and supplied into PLC through the air supply path at a controlled flow rate, and then the air passed through PLC is sampled into CIRAS-1 through the air sampling path for the gas analysis, where this sampled air is exhausted from CIRAS-1 after H_2O and CO_2 gas analyses.

PLC was partitioned into two individual cuvettes for the respective adaxial and abaxial leaf surfaces, i.e. the upper leaf cuvette (U) and the lower leaf cuvette (L). Each cuvette has the air supply path and the air sampling path individually. The rates ($Q_{(U)}$ and $Q_{(L)}$) of air supply to the respective U and L can be adjusted by manipulating the respective flow meters of FM_U and FM_L equipped on the air supply paths. On the air sampling paths for U and L, three -way solenoid valves of SV_U and SV_L were equipped, respectively, which can change the air sampling path and make it possible to select arbitrarily three

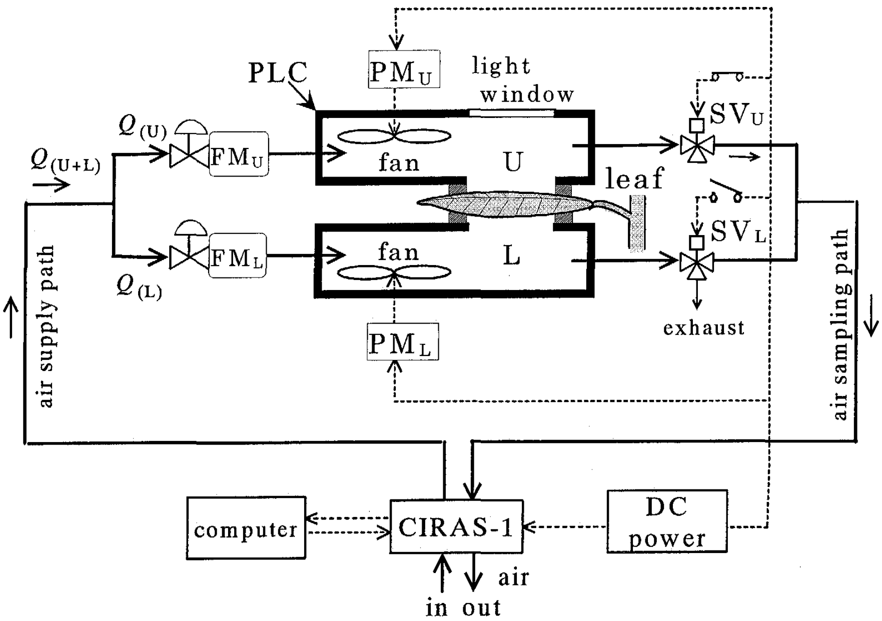

Fig. 1 Schematic diagram of the system for individual evaluations of gas exchange parameters on adaxial and abaxial leaf surfaces under different air currents. The system consists of Parkinson leaf cuvette (PLC) and CIRAS-1 analyzer produced by PP Systems, Ltd. (Hitchin, UK), where new devices are appended to PLC for selecting the three measurement modes, i.e. the upper cuvette mode, the lower cuvette mode and the both cuvettes mode and for independent adjustment of air current on each leaf surface. CIRAS-1 is equipped with CO_2 and H_2O infrared gas analyzers, mass flow controllers and air pumps *etc.* for evaluating leaf gas exchange parameters: FM_L and FM_U, flow meters on the air supply paths for the lower leaf cuvette and for the upper leaf cuvette, respectively; L, lower leaf cuvette; PM_L and PM_U, potentiometers for the microfans in the lower leaf cuvette and in the upper leaf cuvette, respectively; $Q_{(L)}$, $Q_{(U)}$ and $Q_{(UL)}$, the rates of air supply to the lower leaf cuvette, the upper leaf cuvette and the both leaf cuvettes, respectively; SV_L and SV_U, three-way solenoid valves on the air sampling paths from the lower leaf cuvette and from the upper leaf cuvette, respectively; U, upper leaf cuvette; solid line, air flow path; broken line, electric cable. This diagram shows the upper cuvette mode for only adaxial leaf surface, where air sampled from the upper leaf cuvette flows into CIRAS-1 analyzer through SV_U, but air sampled from the lower leaf cuvette is exhausted through SV_L.

modes of measurement (i.e., the upper cuvette mode, the lower cuvette mode and the both cuvettes mode) in succession. The upper cuvette mode can be selected by setting SV_U on and SV_L off, where the air passed through U is sampled into CIRAS-1, but the air passed through L is exhausted as shown in Fig. 1. That is, in the upper cuvette mode (the U mode), the gas exchange parameters are measured only on the adaxial leaf surface. In the similar manner, the lower cuvette mode (the L mode) for the measurement only on the abaxial leaf surface is selected by setting SV_U off and SV_L on, and the both cuvettes mode (the UL mode) for the measurement on the both leaf surfaces is selected by setting both

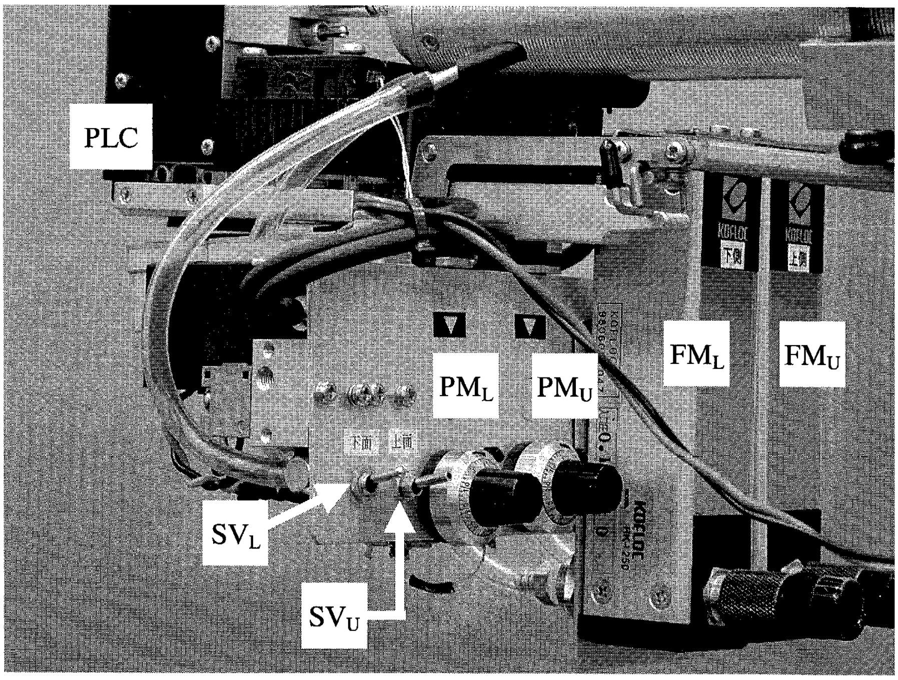

Fig. 2 Photograph of devices newly appended to Parkinson leaf cuvette (PLC) for individual evaluations of gas exchange parameters on adaxial and abaxial leaf surfaces under different air currents: FM_L, FM_U, PM_L, PM_U, SV_L and SV_U are explained in Fig. 1.

SV_U and SV_L on.

Furthermore, the electric power to the microfans for stirring air currents in the respective cuvettes of U and L can be changed by manipulating the newly appended potentiometers (PM_U and PM_L). Therefore, conductance for heat and mass transfer through leaf boundary layer can be adjusted on the adaxial and abaxial surfaces independently. Figure 2 shows a photograph of devices such as flow meters, potentiometers and solenoid valves newly appended to PLC.

EVALUATION

In this system, rates of transpiration and photosynthesis can be evaluated in the general rule for the open system by using air flow rate and respective changes in H_2O and CO_2 gas concentrations through the cuvettes (e.g. *2, 3, 7, 8*). Therefore, transpiration rates ($E_{(U)}$, $E_{(L)}$ and $E_{(UL)}$) in the respective three measurement modes for the adaxial leaf surface, the abaxial leaf surface and the both leaf surfaces can be evaluated by

$$E_{(U)}=Q_{(U)}(e_{out}-e_{in})/(P-e_{out})=Q_{(U)}\Delta H_2O \quad (1a)$$

$$E_{(L)}=Q_{(L)}(e_{out}-e_{in})/(P-e_{out})=Q_{(L)}\Delta H_2O \quad (1b)$$

$$E_{(UL)}=Q_{(UL)}(e_{out}-e_{in})/(P-e_{out})=Q_{(UL)}\Delta H_2O \quad (1c)$$

where $E_{(U)}$ (mol $m^{-2}s^{-1}$) is the transpiration rate on adaxial leaf surface, $E_{(L)}$ (mol $m^{-2}s^{-1}$) is the transpiration rate on abaxial leaf surface, $E_{(UL)}$ (mol $m^{-2}s^{-1}$) is the transpiration rate on both leaf surfaces, e_{in} (hPa) is the water vapor pressure in the supplied air, e_{out} (hPa) is the water vapor pressure in the sampled air, P (hPa) is the atmospheric pressure, $Q_{(U)}$ (mol $m^{-2}s^{-1}$) is the air flow rate through the upper cuvette, $Q_{(L)}$ (mol $m^{-2}s^{-1}$) is the air flow rate through the lower cuvette, $Q_{(UL)}$ (mol $m^{-2}s^{-1}$) is the air flow rate through the both cuvettes (i.e., $Q_{(UL)}=Q_{(U)}+Q_{(L)}$) and ΔH_2O (mol mol^{-1}) is the difference in water vapor concentration between the supplied air and the sampled air. In the similar rule, photosynthetic rates ($A_{(U)}$, $A_{(L)}$ and $A_{(UL)}$) in the respective three measurement modes can be evaluated by

$$A_{(U)}=-\{Q_{(U)}(C_{out}-C_{in})+E_{(U)}C_{out}\}=-(Q_{(U)}\Delta CO_2+E_{(U)}C_{out}) \quad (2a)$$

$$A_{(L)}=-\{Q_{(L)}(C_{out}-C_{in})+E_{(L)}C_{out}\}=-(Q_{(L)}\Delta CO_2+E_{(L)}C_{out}) \quad (2b)$$

$$A_{(UL)}=-\{Q_{(UL)}(C_{out}-C_{in})+E_{(UL)}C_{out}\}=-(Q_{(UL)}\Delta CO_2+E_{(UL)}C_{out}) \quad (2c)$$

where $A_{(U)}$ (μmol $m^{-2}s^{-1}$) is the photosynthetic rate on adaxial leaf surface, $A_{(L)}$ (μmol $m^{-2}s^{-1}$) is the photosynthetic rate on abaxial leaf surface, $A_{(UL)}$ (μmol $m^{-2}s^{-1}$) is the photosynthetic rate on both leaf surfaces, C_{in} (μmol mol^{-1}) is the CO_2 gas concentration in the supplied air, C_{out} (μmol mol^{-1}) is the CO_2 gas concentration in the sampled air and ΔCO_2 (μmol mol^{-1}) is the difference in CO_2 gas concentration between the supplied air and the sampled air.

Stomatal conductances ($G_{S(U)}$, $G_{S(L)}$ and $G_{S(UL)}$) for vapor transfer in the respective three measurement modes can be also evaluated in general rule by using transpiration rates, leaf-to-air vapor pressure difference and leaf boundary layer conductances as

$$G_{S(U)}=\frac{1}{(e_{leaf}-e_{out})/P/E_{(U)}-1/G_{AV(U)}} \quad (3a)$$

$$G_{S(L)}=\frac{1}{(e_{leaf}-e_{out})/P/E_{(L)}-1/G_{AV(L)}} \quad (3b)$$

$$G_{S(UL)}=\frac{1}{(e_{leaf}-e_{out})/P/E_{(UL)}-1/(G_{AV(U)}+G_{AV(L)})} \quad (3c)$$

where $G_{S(U)}$ (mol $m^{-2}s^{-1}$) is the stomatal conductance for vapor transfer on adaxial leaf surface, $G_{S(L)}$ (mol $m^{-2}s^{-1}$) is the stomatal conductance for vapor transfer on abaxial leaf surface, $G_{S(UL)}$ (mol $m^{-2}s^{-1}$) is the stomatal conductance for vapor transfer on both leaf surfaces, e_{leaf} (hPa) is the saturation vapor pressure at leaf temperature, $G_{AV(U)}$ (mol $m^{-2}s^{-1}$) is the leaf boundary layer conductance for vapor transfer on adaxial leaf surface and $G_{AV(L)}$ (mol $m^{-2}s^{-1}$) is the leaf boundary layer conductance for vapor transfer on abaxial leaf surface. The saturation vapor pressure (e_{leaf}) at a leaf temperature (T_L; ℃) was calculated by the following equation *(1, 8)* as

$$e_{leaf}=6.13753\times\exp\{T_L(18.564-T_L/254.4)/(T_L+255.57)\} \quad (4)$$

Based on Eqs (3) and (4), stomatal conductances were evaluated on adaxial leaf surface, abaxial leaf surface and both leaf surfaces individually by using transpiration rates and leaf boundary layer conductances measured on the respective surfaces.

For analysis of effect of air current on leaf gas exchange, it is necessary to determine leaf boundary layer conductances of $G_{AV(U)}$ and $G_{AV(L)}$ under different air currents. Therefore, the relationship between G_{AV} and the electric power applied to the microfan in each cuvette was predetermined by measuring evaporation rate (E_W) from a wetted filter paper under different air currents. G_{AV} on the adaxial surface and the abaxial surface were evaluated by

$$G_{AV(U)} = PE_{W(U)}/(e_{leaf} - e_{out}) \quad (5a)$$

$$G_{AV(L)} = PE_{W(L)}/(e_{leaf} - e_{out}) \quad (5b)$$

where $E_{W(U)}$ is the evaporation rate on upper surface of a wetted filter paper and $E_{W(L)}$ is the evaporation rate on lower surface of a wetted filter paper. Figure 3 shows the relationship between G_{AV} and voltage applied to the microfan in each cuvette. $G_{AV(U)}$ and $G_{AV(L)}$ were increased with increase in the applied voltage and distributed from the minimum of about 0.3 mol $m^{-2} s^{-1}$ to the maximum of about 1.0 mol $m^{-2} s^{-1}$. This suggests that by manipulating the respective

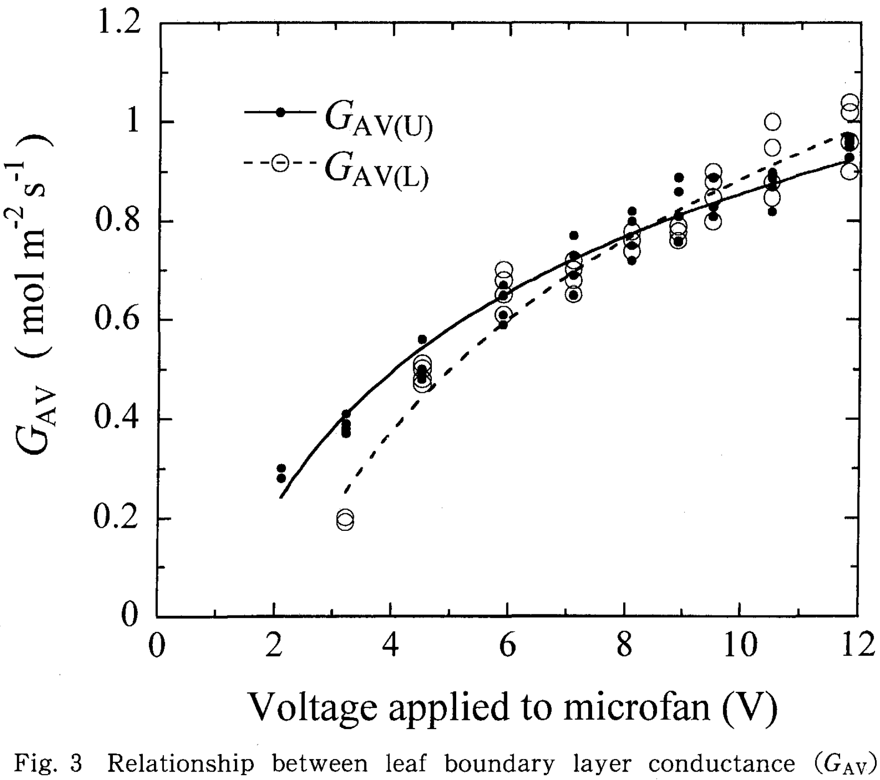

Fig. 3 Relationship between leaf boundary layer conductance (G_{AV}) on each surface and voltage applied to the microfan in each cuvette: $G_{AV(U)}$ and $G_{AV(L)}$, leaf boundary layer conductances for vapor transfer on adaxial and abaxial surfaces, respectively.

potentiometers of PM_U and PM_L, G_{AV} can be adjusted in the range of 0.3 mol $m^{-2} s^{-1}$ to 1.0 mol $m^{-2} s^{-1}$ in the upper and lower leaf cuvettes independently. The dependence of G_{AV} on the applied voltage was somewhat different between the upper and lower cuvettes, and this difference was attributed to different relationship between r.p.m and the applied voltage to the microfans.

PERFORMANCE

Performance of the improved cuvette system was examined in relation to the individual evaluations of gas exchange parameters on adaxial and abaxial leaf surfaces under different air currents. On a wetted filter paper with freely evaporating surfaces and on a fully expanded cucumber leaf with stomata, the measurement mode was changed successively among the three modes of the U mode, the L mode and the UL mode. Furthermore, the potentiometers of microfans in the respective leaf cuvettes were manipulated to change G_{AV}.

Figure 4 shows time course patterns of E_W (i.e., $E_{W(U)}$, $E_{W(L)}$ and $E_{W(UL)}$), ΔH_2O, Q (i.e., $Q_{(U)}$, $Q_{(L)}$ and $Q_{(UL)}$) and G_{AV} (i.e., $G_{AV(U)}$, $G_{AV(L)}$ and $G_{AV(U)}+G_{AV(L)}$) on a wetted filter paper during the successive three measurement modes under different air currents at an air temperature of 25℃ and a saturation vapor deficit of 21 hPa in the dark. The supplied air was divided equally between the upper

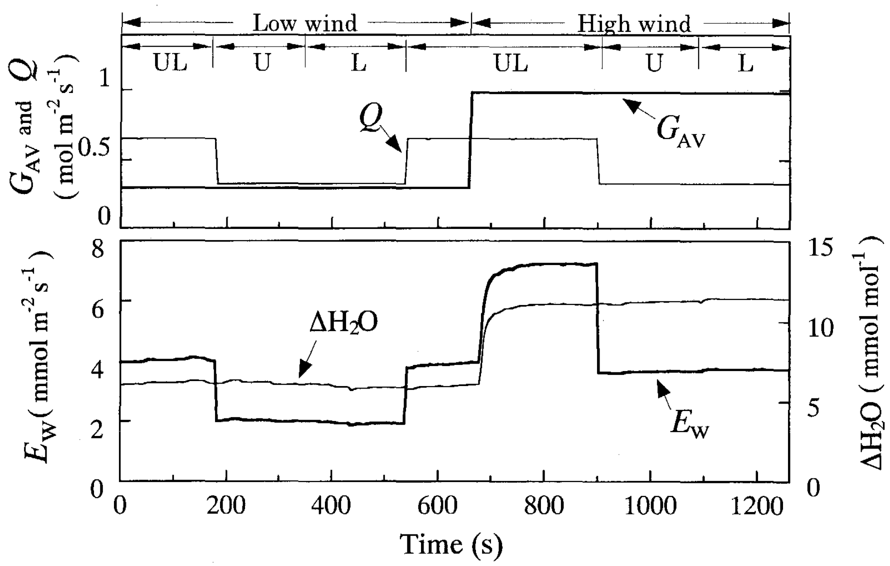

Fig. 4 Time course patterns of evaporation rate (E_W) and variables (G_{AV}, Q and ΔH_2O) used for gas exchange analyses on a wetted filter paper during the successive selection of three measurement modes (U, L and UL) under low and high air currents at an air temperature of 25℃ and a saturation vapor deficit of 21 hPa (about 32% *RH*) in the dark: G_{AV}, leaf boundary layer conductance for vapor transfer; L, the lower cuvette mode; Q, air flow rate through the cuvette in each measurement mode; U, the upper cuvette mode; UL, the both cuvettes mode; ΔH_2O, difference in water vapor concentration between the supplied air and the sampled air.

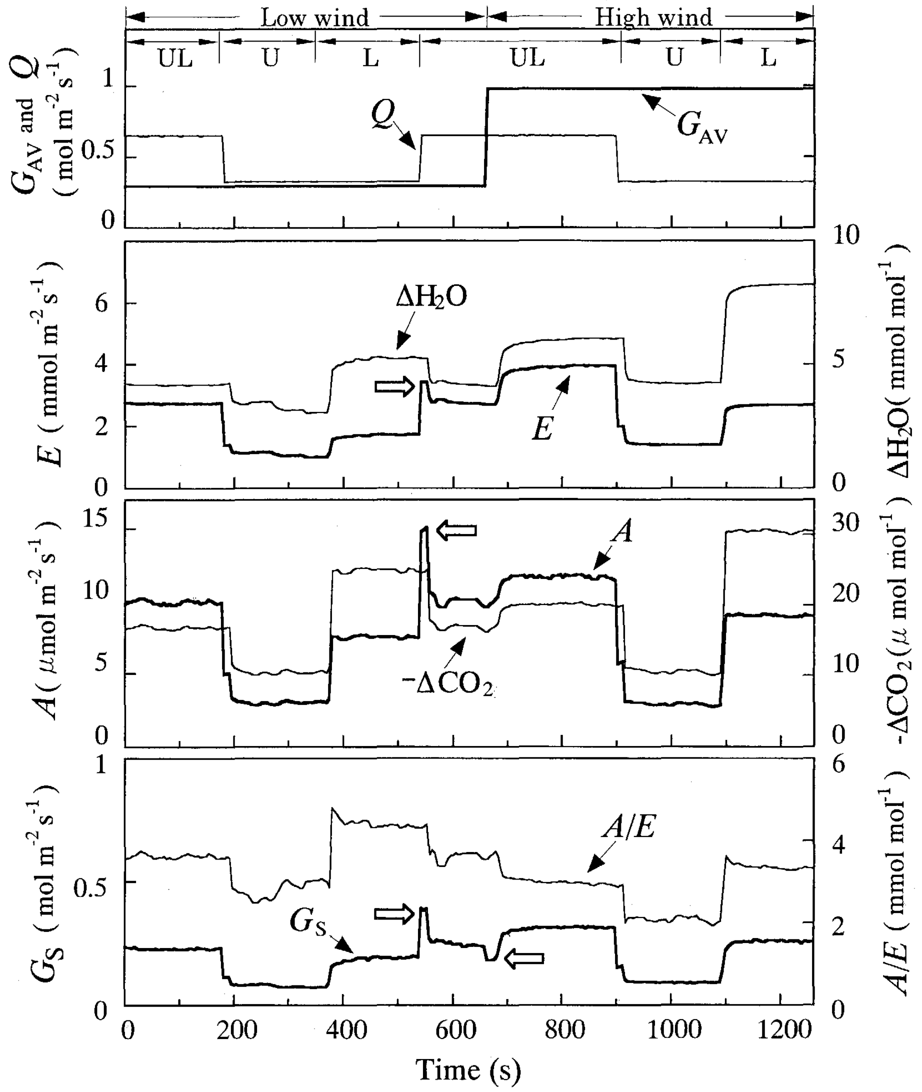

Fig. 5 Time course patterns of transpiration rate (E), photosynthetic rate (A), stomatal conductance for vapor transfer (G_S), water use efficiency (A/E) and variables (G_{AV}, Q, ΔH_2O and ΔCO_2) used for gas exchange analyses on a cucumber leaf during the successive selection of three measurement modes (U, L and UL) under low and high air currents at *PPFD* of 500 μmol m^{-2} s^{-1}, an air temperature of 25℃ and a saturation vapor deficit of 21 hPa (about 32% *RH*) : G_{AV}, leaf boundary layer conductance for vapor transfer; L, the lower cuvette mode; Q, air flow rate through the cuvette in each measurement mode; U, the upper cuvette mode; UL, the both cuvettes mode; ΔCO_2, difference in CO_2 gas concentration between the supplied air and the sampled air; ΔH_2O, difference in water vapor concentration between the supplied air and the sampled air. Open arrows on E, A and G_S indicate transient errors caused by the dead time (about 20 s) in gas sampling and gas analysis with CIRAS-1 when the measurement mode and the air current are changed.

and lower cuvettes by manipulating FM_U and FM_L, respectively, and therefore Q in each of the U mode and the L mode was exactly half of Q in the UL mode. The increment of vapor concentration (ΔH_2O) did not change with the successive switching of the measurement mode under the constant air condition, and therefore E_W in each of the U mode and the L mode was exactly half of E_W in the UL mode. By manipulating PM_U and PM_L, G_{AV} was increased from 0.3 mol $m^{-2} s^{-1}$ to 1.0 mol $m^{-2} s^{-1}$, and E_W in each measurement mode increased by a factor 1.8.

Figure 5 shows time course patterns of E (i.e., $E_{(U)}$, $E_{(L)}$ and $E_{(UL)}$), A (i.e., $A_{(U)}$, $A_{(L)}$ and $A_{(UL)}$), G_S (i.e., $G_{S(U)}$, $G_{S(L)}$ and $G_{S(UL)}$), Q (i.e., $Q_{(U)}$, $Q_{(L)}$ and $Q_{(UL)}$), G_{AV} (i.e., $G_{AV(U)}$, $G_{AV(L)}$ and $G_{AV(U)}+G_{AV(L)}$), ΔH_2O, ΔCO_2 and water use efficiency (A/E) on a cucumber leaf during the successive three measurement modes under different air currents at a *PPFD* of 500 μmol $m^{-2} s^{-1}$, an air temperature of 25℃ and a saturation vapor deficit of 21 hPa. Because of higher stomatal frequency in the abaxial surface of cucumber leaf, E, A and G_S were remarkably higher on the abaxial surface than on the adaxial surface. These differences between the adaxial and abaxial leaf surfaces found to be larger in A, and consequently A/E was also higher in the abaxial surface. This larger difference found in A can be mainly attributed to differences in mesophyll structure and stomatal frequency between adaxial and abaxial sides of the leaf. The increase in G_{AV} from 0.3 mol $m^{-2} s^{-1}$ to 1.0 mol $m^{-2} s^{-1}$ enhanced the leaf gas exchange on both surfaces, and effect of the increase in G_{AV} on stomatal aperture was revealed quantitatively by increase in G_S. This effect of change in air current was larger on the abaxial leaf surface with higher stomatal frequency but found to be less as compared with that in a wetted filter paper without stomatal regulation. In this system, just after changes in the measurement mode and the air current, transient errors (open arrows) were induced by the dead time (about 20 s) in gas sampling and gas analysis with CIRAS-1.

Thus, the improvement of Parkinson leaf cuvette enabled quantitative and individual evaluations of gas exchange on adaxial and abaxial leaf surfaces under different air currents, and the improved cuvette system might be applicable to studies on stomatal response to air current and scaling-up of gas exchange parameters over heterogeneous surfaces.

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