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出版情報：BIOTRONICS. 29, pp.71-78, 2000-12. Biotron Institute, Kyushu University

BIOTRONICS **29**, 71–78, 2000

METHODOLOGICAL ESTABLISHMENT FOR INDIVIDUAL EVALUATIONS OF HYDRAULIC CONDUCTANCES OF NODE, INTERNODE AND LEAF INSERTION IN A NODAL COMPLEX

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(Received September 25, 2000; accepted November 20, 2000)

NAGASUGA K., YASUTAKE D., ARAKI T., and KITANO M. *Methodological establishment for individual evaluations of hydraulic conductances of node, internode and leaf insertion in a nodal complex.* BIOTRONICS **29**, 71–78, 2000. A pressure flow meter system was newly developed for measuring hydraulic characteristics of plant organs. In the system, the pressurized water flow through plant organs was given at a desired rate by regulating the applied pressure, and hydraulic resistance or conductance of plant organs was determined from the flow rate and the applied pressure at intervals of about 5 minutes. By applying the system, individual evaluations of hydraulic characteristics of node, internode and leaf insertion in a nodal complex were performed on the basis of an analogue circuit of hydraulic resistances, where the hydraulic resistance of the node was divided into halves at the leaf insertion point. From measurements with *Epipremnum aureum* Bunt., *Cucumis sativus* L. and *Zea mays* L., it was indicated that hydraulic conductance of a leaf insertion was significantly lower than those of node and internode. *Z. mays* with the thickest stem had the highest hydraulic conductance of the internode. On the other hand, the stem-area specific hydraulic conductances of node and internode were the highest in *C. sativus* with the thinnest stem and the large leaf.

Key words: hydraulic conductance; pressure flow meter; nodal complex; herbaceous plants.

INTRODUCTION

The movement of liquid phase water through a plant body is primarily restricted by roots, whereas the vapor phase movement is definitely controlled by stomata. Hydraulic conductance of water pathway components is one of the main factors controlling the movement of water from roots to the atmosphere, influencing water use efficiency and photosynthesis in cultivated crops. Generally, the conductivity for water flow in xylem is greatly high in stem (*2*), and hence this parameter is not regarded as a restricting factor for water movement through a plant body. However, in large herbaceous plants, such as sugarcane and napiergrass plants, their stems with nodes are also likely to play an important role in regulation of water movement through their bodies (*4, 5*).

Hydraulic conductance of plants is generally measured by the evaporative flux method (EF method) or by the high pressure flow meter method (HPFM method). The EF method, which has been frequently used for a whole plant, evaluates the hydraulic conductance on the basis of the linear relationship between steady-state evaporative flux and water potential difference between soil and leaves. However, the linear relationship between these parameters is not always obtained, because hydraulic conductance of a whole plant increases nonlinearly with rise in evaporative flux in a certain case (*3*). On the other hand, HPFM method can be applied to individual plant organs as well as a whole plant, where the water pressure applied to the plant specimen and the rate of quasi-steady water flow can be measured. HPFM method always allows a rapid establishment of a linear relationship between the applied pressure and the flow rate (*4, 6, 7, 8, 9*), which enables a rapid determination of the hydraulic conductance of individual plant organs. However, HPFM method has been mainly used for woody plants, not so many times for herbaceous plants (*4, 8, 9*). Therefore, only limited information is available for hydraulic conductance of herbaceous plant organs.

The present study deals with a newly developed system for the pressure flow meter method applicable to herbaceous plants, and its performance was examined in rapid and individual evaluations of hydraulic conductances of node, internode and leaf insertion in a nodal complex of three herbaceous plants.

METHODS AND MATERIALS

System

A pressure flow meter was newly developed for measuring hydraulic resistance or conductance of individual plant organs such as roots, stem and leaves, where pressurized water flow was differently applied to these organs with different pressure. Figure 1 shows a schematic diagram of the pressure flow meter. The system consists of a water pressurizing unit, a pressure regulating unit and detectors of pressure and flow rate. In the water pressurizing unit, high pressure nitrogen gas was introduced into a pressurized water tank (PWT) of 2 L in volume. Nitrogen gas pressure was coarsely adjusted by a pressure regulator (PR_N), and the pressurized water flow out of PWT was finely readjusted to a desired pressure by the two pressure regulators of PR_L and PR_H, by which low pressure and high pressure were controlled, respectively. The excessive pressure was discharged through an exhausting valve. The flow rate of adequately pressurized water was measured by a mass flow meter (MFM, Model 5892, Brooks Instrument B. V., the Netherlands) with an accuracy of $\pm$ 0.3% F.S. The water pressure was measured with an accuracy of $\pm$ 1 % F.S. by the low pressure transmitter of PT_L (KH-15-B-33, NAGANO, Japan) for the pressure range lower than 0.1 MPa or by the high pressure transmitter of PT_H

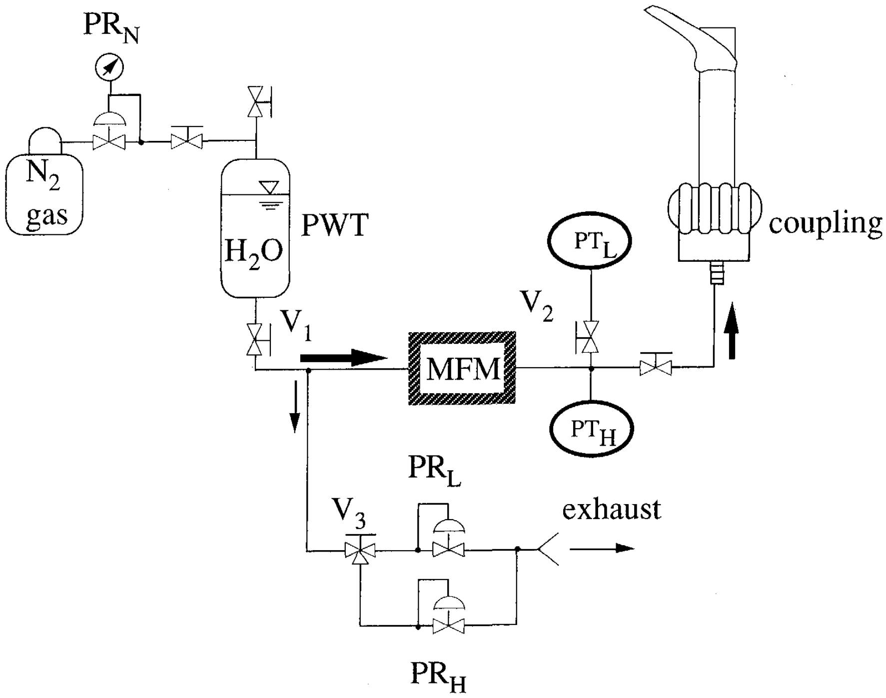


Fig. 1. Schematic diagram of a pressure flow meter newly developed for a measurement of plant hydraulic characteristics: MFM, mass flow meter; PT_H, high pressure transmitter; PT_L, low pressure transmitter; PR_H, high pressure regulator; PR_L, low pressure regulator; PR_N, Nitrogen gas pressure regulator; PWT, pressurized water tank; V_1, valve for PWT; V_2, valve for the low pressure transmitter; V_3, valve for the pressure regulator.

(KH-15-B-33, NAGANO, Japan) for the pressure range higher than 0.1 MPa. The pressurized water flow was applied to a cut end of a plant organ through a water tight coupling (HPLC coupling, Dynamax, USA). In this system, the applied pressure and the water flow rate was monitored on a computer system or an analog recorder, and the desired water flow rate was easily adjusted by regulating the applied pressure in a wide range of 0.01 MPa to 10 MPa.

Evaluation

To evaluate hydraulic resistance or conductance of node, internode and leaf insertion in a nodal complex individually, an analogue circuit of resistances was assumed for water flow through a nodal complex as shown in Fig. 2. Hydraulic resistance of leaf insertion was assumed to locate on the half position of the node, and consequently the resistance of the node was divided into two halves at the joint between the leaf insertion and the node.

Hydraulic resistance of each component in the analog circuit of a nodal complex was determined through the successive three procedures with Case 1, Case 2 and Case 3 as shown in Fig. 3. In Case 1, water flow through the upper half of the node was inhibited by sealing the apical end of the node with an adhesive agent of cyanoacrylic glue, and the complex resistance (R_1) for water

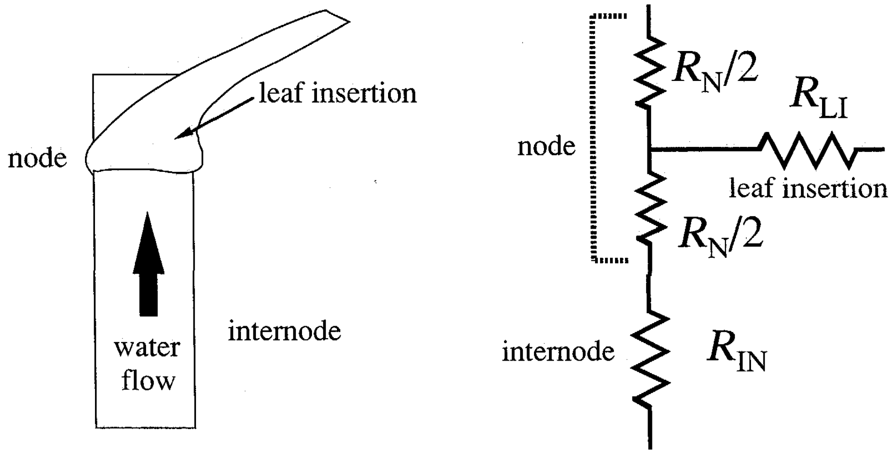
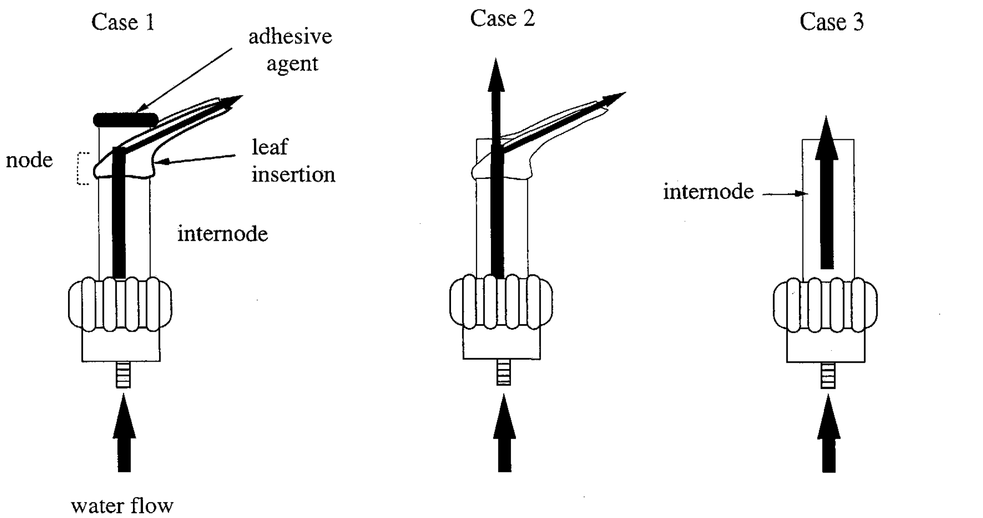


Fig. 2. An analogue circuit of hydraulic system for a nodal complex composed of a node, an internode and a leaf insertion: R_N, hydraulic resistance of a node; R_{IN}, hydraulic resistance of an internode; R_{LI}, hydraulic resistance of a leaf insertion. R_N was assumed to be divided into two halves at the joint between leaf and node.



$$R_1=R_{IN}+R_N/2+R_{LI} \cdot\cdot\cdot [1] \quad R_2=R_{IN}+R_N/2+\frac{R_N/2\times R_{LI}}{R_N/2+R_{LI}} \cdot\cdot\cdot [2] \quad R_3=R_{IN} \cdot\cdot\cdot [3]$$

Fig. 3. Schematic diagram of experimental procedures for determining the respective hydraulic resistances of node (R_N), internode (R_{IN}) and leaf insertion (R_{LI}) in a nodal complex: R_N, R_{IN} and R_{LI} were determined by solving Eqs [1]–[3] for the complex resistances of R_1, R_2 and R_3, which were measured in the successive three procedures with the respective Case 1, Case 2 and Case 3.

flow in Case 1 can be given by

$$R_1=R_{IN}+R_N/2+R_{LI} \qquad [1]$$

where R_{IN}, R_N and R_{LI} are the hydraulic resistances of internode, node and leaf insertion, respectively.

In Case 2, the adhesive agent on the apical end of the node was removed, and the pressurized water was passed through both the leaf insertion and the apical end of the node. Therefore, the complex resistance (R_2) for the water flow in Case 2 can be given by

$$R_2=R_{IN}+R_N/2+(R_N/2\times R_{LI})/(R_N/2+R_{LI}) \quad [2]$$

In Case 3, the node was excised at its basal end, and the pressurized water flowed through only the segment of internode. Consequently, the resistance (R_3) for water flow in Case 3 is equal to the resistance of internode as

$$R_3=R_{IN} \quad [3]$$

The respective resistances for internode (R_{IN}), node (R_N) and leaf insertion (R_{LI}) were determined by solving Eqs. [1], [2] and [3] simultaneously. The hydraulic conductance can be given as the reciprocal of resistance. That is, the respective conductances for internode (K_{IN}), node (K_N) and leaf insertion (K_{LI}) were calculated by $K_{LI}=1/R_{LI}$, $K_N=1/R_N$ and $K_{LI}=1/R_{LI}$. Furthermore, leaf-area specific hydraulic conductance (k_{LI}) for leaf insertion was evaluated by dividing K_{LI} by area (LA) of a leaf attached to the node (i.e., $k_{LI}=K_{LI}/LA$). Similarly stem-area specific hydraulic conductance for internode (k_{IN}) and node (k_N) were evaluated by $k_{IN}=K_{IN}/SA$ and $k_N=K_N/SA$, respectively, where SA was the cross-sectional area of the internodal stem. In this study, the node was defined as the region within 5 mm apart from the leaf insertion point.

Plant materials

Nodal complexes were sampled from well-grown shoots of different three species of *Epipremnum aureum* Bunt., *Cucumis sativus* L. and *Zea mays* L. Three nodal complexes at a similar growth stage were used for experiments with the respective species. Leaves were excised near the node and stem segments were 5 cm to 10 cm in length.

RESULTS AND DISCUSSION

Figure 4 shows typical time course patterns of water flow rate and applied pressure during the successive three experimental procedures with Case 1, Case 2 and Case 3 explained in Fig. 3. The applied pressure was adjusted by the pressure regulating unit to set the water flow rate at the level similar to the daytime transpiration rate (i.e., 0.2–0.5 ml min^{-1} in Case 1 and 1.0–2.0 ml min^{-1} in Cases 2 and 3). The nodal complex was supplied with water at a pressure of about 100 kPa in Case 1, about 30 kPa in Case 2 and about 10 kPa in Case 3. In each case, it took about 5 minutes for water flow rate to become stable after changing the applied pressure. The complex hydraulic resistances (i.e., R_1, R_2 and R_3) in the respective procedures with Case 1, Case 2 and Case 3 were 163.8 kPa s mmol^{-1}, 26.19 kPa s mmol^{-1} and 5.663 kPa s mmol^{-1}.

Table 1 shows K_N, K_{IN} and K_{LI} of *E. aureum, C. sativus,* and *Z. mays.*

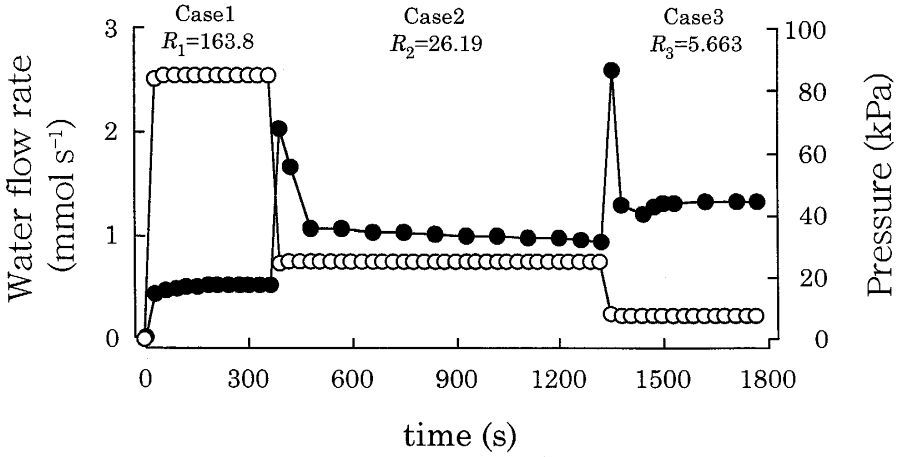


Fig. 4. Time courses of applied pressure (○) and water flow rate (●) in the successive three procedures with Case 1, Case 2 and Case 3 explained in Fig. 3. The complex hydraulic resistances of R_1, R_2 and R_3 (kPa s mmol^{-1}) were evaluated by dividing the applied pressure by the water flow rate in Case 1, Case 2 and Case 3, respectively.

Table 1. Hydraulic conductances of node (K_N), internode (K_{IN}) and leaf insertion (K_{LI}) in a nodal complex of three plant species.

species	hydraulic conductance (mmol s^{-1} MPa^{-1})		
	K_N	K_{IN}	K_{LI}
Epipremnum aureum	16.6±6.00	4.68±1.36	0.22±0.09
Cucumis sativus	104±26.8	72.7±14.4	2.63±0.34
Zea mays	41.5±15.3	456±182	4.40±1.05

Values are means±SE (n=6).

Although the magnitude of hydraulic conductance was quite varied with species, K_{LI} was the lowest in all the three species. This lowest conductance of the leaf insertion can be considered to result from hydraulic constriction and divergence at the leaf insertion point (*9*), which induced remarkable decrease in xylem water potential (*1, 9*). This would tend to confine formation of cavitation and embolism to expendable leaves, resulting in conservations of water flux in the stem without serious damage by water deficit (*1, 4*). Furthermore, it is notable that the remarkably larger K_{IN} was found in *Z. mays* which had the thickest internode among the three species. This suggests that the *Z. mays* stem has the largest capacity for water transport.

It can be considered that hydraulic characteristics of plant organs are presented more explanatory in terms of specific hydraulic conductance (i.e., k_{LI}, k_{IN} and k_N). Table. 2 shows k_{LI} and LA in three plant species. The relationship among three species in k_{LI} was similar to that in K_{LI}, but the difference among

Table 2. Leaf-area specific hydraulic conductance (k_{LI}) of leaf insertion and area of the leaf attached to the node in three plant species.

species	k_{LI} ($\times 10^2$ mmol s^{-1} MPa^{-1} m^{-2})	leaf area (cm^2)
Epipremnum aureum	0.46±0.25	50.6±5.79
Cucumis sativus	0.85±0.08	305±22.9
Zea mays	1.23±0.39	361±42.1

k_{LI} was determined by dividing the conductance (K_{LI}) for leaf insertion by the leaf area. Values are means±SE (n=5 or 6).

Table 3. Stem-area specific hydraulic conductances of node (k_N) and internode (k_{IN}), and diameter of the internodal stem in three plant species.

species	k_N (mol s^{-1} MPa^{-1} m^{-2})	k_{IN} (mol s^{-1} MPa^{-1} m^{-2})	diameter (mm)
Epipremnum aureum	212±0.80	55.2±11.5	5.06±0.34
Cucumis sativus	1111±452	934±291	4.99±0.20
Zea mays	46.2±21.1	495±218	17.9±0.57

k_N and k_{IN} were determined by dividing the respective conductances (K_N and K_{IN}) of node and internode by the cross-sectional area of the internodal stem. Values are means±SE (n=4 or 6).

the species was smaller in k_{LI}. Table. 3 shows k_N, k_{IN} and SA in three plant species. The highest k_N and k_{IN} were found in the nodal complex of *C. sativus* plant, and this suggests that the thin stem of *C. sativus* can transport enough water for transpiration in its large leaves. Although *E. aureum* as well as *C. sativus* had the thin stem, k_N and k_{IN} in *E. aureum* were not so high reflecting its smallest leaves. There found to be species specificity but no clear correlation between hydraulic conductance and stem diameter. For further understanding of hydraulic characteristics of the nodal complex, it seemed necessary to analyze anatomical properties of vascular system and hydraulic characteristics of the nodal complex exposed to different environmental conditions such as water stress. These results suggest that the HPFM system newly developed can be applied to rapid and individual evaluations of hydraulic characteristics of node, internode and leaf insertion in a nodal complex of herbaceous plants.

Acknowledgment

This work was supported by a Grant-in-aid of Scientific Research (No. 11460122) from the Ministry of Education, Science, Sports and Culture, Japan.

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