Superposition of the Transpiration-induced Water Potential and the Growth-induced Water Potential Associated with Expanding Tomato Leaves

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(Received June 7, 2010)

The growth parameters of Lockhart’s equation were determined in expanding leaves of tomato plants (Solanum lycopersicum L.) subjected to different relative humidity in the dark. In tomato leaves, sizes of the growth-induced water potential exhibited linear relations with the relative growth rates (RGR) under various vapor pressure difference (VPD) conditions between leaves and the ambient air. When transpiration rates of tomato plants became significantly larger, lines formed between the growth-induced water potential and RGR had a translational relation as VPD increased. Then, the transpiration-induced water potential became proportionally larger as VPD increased. These results indicated that transpiration flux and growth flux have additive relations, suggesting that water fluxes for growth and transpiration are linearly superimposed.

**Keywords**: cell wall extensibility, hydraulic conductivity, Solanum lycopersicum L., transpiration, turgor

**INTRODUCTION**

When cell elongation and transpiration occur simultaneously in plants, it is possible that water absorption due to transpiration may alter water flux into expanding cells. If the water flux for cell expansion and transpiration moves along the same path, water potential gradients for both processes will be additive. However, some researchers pointed out that the growth-induced water potential is obscured by the transpiration-induced water potential when significant transpiration occurs (Boyer, 1974; Westgate and Boyer, 1984). Westgate and Boyer (1984) demonstrated that there is an effect on the growth-induced water potential as the transpiration-induced water potential develops within all vegetative tissues of maize. The interaction between the transpiration-induced water potential and the growth-induced water potential has not been critically examined in actively growing and relatively mature leaves under different vapor pressure difference (VPD) conditions between leaves and the ambient air.

In this paper, we investigated i) whether transpiration alters growth rates of tomato leaves, and ii) whether the water pathway for leaf growth and transpiration is identical in tomato plants grown under various humidity conditions. Water relations parameters were analyzed by applying Lockhart’s equation to transpiring leaves as Ikeda et al. (1999) applied to the tissue-cultured tis-

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The mechanisms of cell enlargement of plant cells require high enough turgor to extend the cell walls and the persistent differences in water potential between growing tissue and the water source to provide water for the enlargement process. The steady relative growth rate (\( G; \) unit: \( \text{s}^{-1} \)) of tissue enlargement can be related to the averaged wall extensibility (\( m; \) unit: \( \text{s}^{-1} \text{MPa}^{-1} \)) and the averaged turgor (\( \Psi_p; \) unit: MPa) that extends the cell walls outward by the following equation (Green et al., 1971):

\[
G = m(\Psi_p - Y)
\]

where \( Y \) (unit: MPa) is the yield threshold turgor below which the force on the wall is too small to enlarge the wall irreversibly. Thus, \( (\Psi_p - Y) \) is the growth-effective turgor.

In many growing tissues, the persistent differences in water potential between growing tissue and the water source were observed in elongating stems (Cavaliere and Boyer, 1982; Nonami and Boyer, 1987, 1990; Ikeda et al., 1999), expanding leaves (Michelena and Boyer, 1982; Fricke and Flowers, 1998), root apices (Sharp and Davies, 1979) and developing grain (Barlow et al., 1980). Such a water potential field associated with cell elongation is called the growth-induced water potential (Molz and Boyer, 1978; Boyer, 1985; Nonami and Boyer, 1987; Nonami et al., 1997). Nonami and Boyer (1993) indicated that the existence of water potential field associated with growth was demonstrated by measuring single cell water potentials of an actively growing soybean stem. The steady rate of water uptake necessary to support cell enlargement can be determined by the average hydraulic conductance (\( L; \) unit: \( \text{s}^{-1} \text{MPa}^{-1} \)) and the growth-induced water potential (\( \Psi_p - \Psi_e \)) obtained from average water potential difference between elongating cells (\( \Psi_e \)) and the water source (\( \Psi_e \)). The relationship can be expressed as:

\[
G = L(\Psi_e - \Psi_p)
\]

As water uptake and cell wall extension simultaneously occur, Equations 1 and 2 can be combined by the relation of \( \Psi_e = \Psi_o + \Psi_p \), assuming that water potential in cytoplasm is locally equilibrated with that in the wall space (Molz and Boyer, 1978; Nonami and Boyer, 1987). Hence,

\[
G = \frac{mL}{m+L}(\Psi_o - \Psi_p - Y)
\]

This equation is known as Lockhart’s combined equation governing cell enlargement (Lockhart, 1965a, 1965b) and showing both the effects of wall extensibility and water conductance. Since it is difficult to determine all parameters in Equation 3 simultaneously, Ikeda et al. (1999) rearranged Equation 3 as follows:

\[
\frac{G}{m} + \frac{G}{L} = (\Psi_o - \Psi_e) + (\Psi_p - Y)
\]

This equation is shown that parameters of \( (\Psi_o - \Psi_e) \) and \( (\Psi_p - Y) \) are linearly separated.

This work was undertaken to make it clear whether the growth-effective turgor and the growth-induced water potential respond to expanding leaves under transpiration.

**MATERIALS AND METHODS**

Tomato seeds (\( S. lycopersicum \) L. cv. Momotaro) were disinfected with 5% NaOCl solution for 15 min, and rinsed with distilled water. Tomato seeds were germinated in a petri dish at 25 ± 1°C. After germination, they were sown in vermiculite grown for 6 days by fertilizing with 0.15 S·m⁻¹ of hydroponic solution (Otsuka House Fertilizer, Otsuka Chemical Co., Ltd., Osaka, Japan). Subsequently, the seedlings were grown hydroponically. The concentration of the
GROWTH OF TOMATO LEAVES

hydroponic solution was adjusted to 0.15 S·m⁻¹ that is equivalent to −0.07 MPa of water potential. Tomato plants were grown for one month prior to the beginning of the experiment in the greenhouse under natural sunlight. For leaf growth experiment, when tomato plants reached about 40 cm high, which produced the twelfth or thirteenth leaf, they were used in the experiment. Before the experiment, tomato plants were transferred in a growth chamber and acclimated for 24 h before measuring in dark.

Growth measurement

In order to compare with the distribution of growth rates between the expanding leaves (leaflet of the 11th leaf) and the mature leaves (leaflets of the 5th leaf), leaves were marked with an India ink at 5-mm intervals using a fine brush. These points and the lines of leaf were copied on the OHP film, the OHP film was copied on a sheet of paper and the leaf area of each plot was calculated. The extension between the marked positions was measured and relative growth rates (RGR) were calculated at each marked position for 24 h of growth. The elongation rates of tomato leaves for 24 h in dark were measured as the increase in elongation between marked positions using a digital caliper.

Control of relative humidity

Tomato plants were transplanted in a growth chamber that the inside RH could be regulated with a humidifier and a dehumidifier. A tomato plant in a pot was put on an electric balance having 0.1 g sensitivity (Sartorius MC1, Sartorius AG, Gottingen, Germany) to measure transpiration rates. During measurements of transpiration rates, vapor pressure difference between tomato leaves and the surrounding air was set at 0 kPa (i.e., 100% RH, 25°C), 0.63 kPa (i.e., 80±5% RH, 25°C), 1.27 kPa (i.e., 60±5% RH, 25°C) and 1.74 kPa (i.e., 45±5% RH, 25°C) conditions in hydroponic culture in the dark in order to minimize the effect of light on transpiration in plants. The saturated vapor pressure of water at 25°C is 3.17 kPa (23.756 mmHg). Chamber RH was monitored with a small probe (Hygrotest, model testo 6400, Lead Electric Inc., Osaka, Japan) with a sensitivity of 0.1% RH in ranges between 15 and 98% RH at 25°C. Under 100% RH-setting condition, water was logged at the bottom of the chamber. Since dew formation occurred at the surface of the sensor, the humidity sensor could not be used for RH measurements. Thus, the air in the chamber should be saturated with water vapor. The top of the pot was covered with a plastic film to retard evaporation from the hydroponic solution. The transpiration rates were measured by the loss of water from the total mass of the pot, hydroponic solution, and plant. The balance was placed in a controlled-temperature room (25±1°C) for all experiments. Leaf area of the plant was measured by copying individual leaves with a copy machine, shapes of leaves were cut out from the copied paper, and the weight of the cut papers were measured with an electric balance. Then, the leaf area was calculated from the weight per unit area of the copied paper.

Water status of measurement with a psychrometer

After the RGRs of leaves were determined, their water status was measured at the same position by using the isopiestic psychrometer (Boyer and Knipling, 1965). This measurement prevented errors caused by the diffusive resistance of the tissue to water vapor since no net vapor exchange took place during the course of the measurement. A thermocouple chamber was coated with melted and resolidified petrolatum due to minimize water absorption, and loaded with plant tissues immediately after excision. After the water potential (ψw) was measured, the osmotic potential (ψs) of the same tissue was immediately determined by the isopiestic psychrometer after freezing at −70°C to rupture cell membranes and thawing. The turgor (ψt) was calculated by subtracting the osmotic potential from the water potential (Nonami et al., 1987).

RESULTS AND DISCUSSION

While leaf expansion was occurring in tomato plants grown hydroponically in a greenhouse,
RGRs were measured in various locations of an actively expanding leaf (the eleventh leaflet) and a relatively mature leaf (the fifth leaflet). In young expanding leaves, the growing region was located in the edge of the leaf, and in relatively mature leaves, the growth region was limited around the central region along the midrib (Fig. 1A). Sizes of water potentials measured with the psychrometer in leaves were negatively correlated with those of RGRs and distributed similarly in the equipotent lines (Figs. 1A and 1B). When the water potential and the corresponding RGRs were plotted, the linear relations were obtained (Fig. 1C). This indicates that persistent water potential gradients existed between the expanding leaves and the water source.

While the plants were dehydrated after withdrawing the hydroponic solution from the root zone, the apoplast solution of growing leaves was extracted with the pressure chamber after sealing the leaf inside the chamber, and the osmotic potential of the apoplast was −0.1 MPa as the average, ranging from −0.07 to −0.13 MPa. Also, the water potential of the hydroponic solution (Ψw) in Fig. 2B was similar to the osmotic potential of the apoplast solution even though plants were dehydrated. Thus, it is safe to assume that the water potential of the water source in leaves was almost constant even transpiration rates were modified due to changes in air humidity. Additionally, effects of transpiration on changes in the apoplast osmotic potential were minimized by conducting the experiment in the dark.

In order to investigate the effect of transpiration on the leaf water status, the water status of transpiring leaves was compared with that of non-transpiring leaves. Under dark condition, plant transpiration were maintained at 0.06 mmol·m⁻²·s⁻¹ at 0.63 kPa VPD, 0.12 mmol·m⁻²·s⁻¹ at 1.27 kPa VPD and 0.16 mmol·m⁻²·s⁻¹ at 1.74 kPa VPD (Fig. 3A). The water status and the corresponding RGR were measured in plants exposing at 1.27 kPa VPD and 1.74 kPa VPD and compared with those of non-transpiring leaves (0 kPa VPD) (Fig. 3). When tomato leaves were expanding rapidly, the water potential of leaves became significantly lower than the water potential of the culture solution (Fig. 3B). Transpiring leaves grown at 1.27 kPa VPD and 1.74 kPa VPD had lower water

**Fig. 1** Distribution of the relative growth rates (A) and water potentials (B) in the top-end leaflet of the 11th expanding leaf and 5th mature leaf, and the relationship between the RGR and water potential of leaves sampled from the mature 5th leaves and expanding 11th leaves of tomato plants (C). Each symbol (□, ■, ○ and △) indicates the sampling positions.
Fig. 2  Turgor (A), apoplast solute potential (Ψₑ, exudate), water potential (Ψᵢ) and osmotic potential (Ψₒ) measured with isosiphasic psychrometers against the matric potential measured with the pressure chamber in tomato leaves (B). Opened triangle (△), opened circle (○) and closed circle (●) indicate exudate water potential, water potential and osmotic potential, respectively.

potentials than non-transpiring leaves at 0 kPa VPD (Fig. 3B).

When the growth-induced water potential was obtained by calculating the difference between water potentials of various growing region and the water source, the size of the growth-induced water potential exhibited a linear relation with RGRs in tomato leaves (Fig. 4). As the size of the growth-induced water potential became larger, the RGR became proportionally larger in each VPD condition. In case of 0 kPa VPD, the regression line could be formed through the origin, i.e., \( y = 0.774 \times x \) and \( r = 0.899 \) when the slope of line at 1.27 kPa VPD was assumed to have the same slope as at 0 kPa VPD, the regression line was translated along the x-axis, i.e., \( y = 0.774 \times x - 0.0681 \) and \( r = 0.955 \). Similarly, the regression line for 1.74 kPa VPD was \( y = 0.774 \times x - 0.1412 \) and \( r = 0.826 \). The x-intercepts for 1.27 kPa VPD and 1.74 kPa VPD were 0.09 MPa and 0.18 MPa, respectively (Fig. 4). The Working-Hotelling hyperbolic confidence bands for the regression lines were calculated from data points shown in Fig. 4 at \( P = 0.99 \) (Neter et al., 1983) and are drawn along the regression lines (Fig. 4). It is considered that regression lines drawn in regions sandwiched between the hyperbolic curves can exist at the same probability, and thus, the assumption used to calculated the regression lines in Fig. 4 by fixing the slope can be rationalized. If the regression line for 1.27 kPa VPD is calculated directly from data points, it will be \( y = 0.799 \times x - 0.0733 \) (\( r = 0.970 \)). Thus, the line is contained within the confidence band. Since transpiration rates were increased proportionally as VPD increased (Fig. 3A), it can be concluded that the shift of lines along the x-axis was caused by the corresponding decrease in water potential in growing leaves due to transpiration. Because the slope of each line was the same, hydraulic conductance associated to the growth process should be the same, and thus, in the present experiment, transpiring process did not interfere directly with water flow associated to cell expansion. Therefore, it is
Fig. 3  Transpiration rates of tomato plants at different vapor pressure differences (VPD) between the leaf and its ambient air in the dark ($y = 0.0945x; r^2 = 0.999$) (A), and water potential and osmotic potential (B) of tomato leaves plotted against the RGR of the leaves when the plants were grown under 0 kPa VPD (○), 1.27 kPa VPD (■) and 1.74 kPa VPD (△) at 25±1°C in the dark. A horizontal dashed line indicates the water potential of the culture solution ($\Psi_c$).

evident that decreases in water potential due to transpiration were superimposed over the growth-induced water potential.

When RGRs were plotted against turgor of expanding leaves, turgor was almost similar under all VPD conditions despite having various water potentials and osmotic potentials (Figs. 5A, 5C and 5E). Because $m$ must be positive for the validity of equation 1, the $m$ was considered to be infinitely large, i.e., $m = +\infty$ (Figs. 5B, 5C and 5F). Although $Y$ should be smaller than turgor according to Equation 1, the effective-turgor was considered negligibly small in the present experiment. This indicates that the effective-turgor was not a limiting factor for growth in this case. Values of $Y$ were obtained by averaging turgor, and the intercept at $G = 0$ was used to estimate the $Y$ in this case (Figs. 5A, 5C and 5E). From the intercept at the x-axis, the $Y$ in 0 kPa VPD, 1.27 kPa VPD, and 1.74 kPa VPD was estimated to be 0.61 MPa, 0.57 MPa, and 0.52 MPa, respectively (Figs. 5A, 5C, and 5E). When transpiration rates increased under lower humidity, values of $Y$ tended to decrease slightly.

Growth in tomato leaves is over within several days after differentiation. When leaf growth and vascular differentiation were studied, the growth-induced water potential was found in *Festuca arundinacea* leaves (Martre et al., 2001). In elongating leaves, immature xylem in the basal growing region led to low hydraulic conductivity (Martre et al., 2001). Nonami et al. (1997) showed in growing soybean hypocotyls that the major resistance to water flow lies in the small cell..
surrounding the protoxylem vessels. It is most likely that the growth-induced water potential in tomato leaves is associated with underdeveloped vascular systems in expanding leaves. Once vascular systems are established and matured, growth of tomato leaves becomes negligibly small, and the water potential gradients in leaves disappear as shown in Fig. 1.

In this study, the size of turgor was not directly related with the RGR in tomato leaves under all RH treatments (Figs. 5A, 5C and 5E). The $Y$ of tomato leaves estimated in case of $m = +\infty$ tended to become slightly greater as RH was elevated. Cutler et al. (1980) demonstrated that there was no apparent change in $Y$ when rice was subjected to a series of drying cycles. Some reports (Van Volkenburgh and Cleland, 1986; Matthews et al., 1984) showed $Y$ could change in response to the leaf age, to humidity, or water-deficit stress, but a change in $Y$ was small. Similarly, the changes in $Y$ obtained from tomato leaves ranged between 0.52 MPa at 1.74 kPa VPD and 0.61 MPa at 0 kPa VPD.

In order to keep the osmotic potential of the apoplast solution in expanding leaves similar to the water potential of the hydroponic solution under transpiring conditions, measurements of transpiration and leaf growth were conducted in the dark in the present study. Under such a condition, transpiration rates had a linear relation with VPD although stomata might not open completely in the dark (Fig. 3A). Because transpiration rates were kept relatively low in the dark, growth rates of leaves could be measured even at 1.74 kPa VPD although reduction in growth was apparent, compared with plants kept at 0 kPa and 1.27 kPa VPD (Figs. 3B and 4).

Since the osmotic potential of xylem solution in tomato leaves was higher than $-0.1$ MPa, it was found that the apoplast solution contained low concentrations of solutes in leaves. Therefore, it is safe to say that the water potential of transpiring tomato leaves was mostly consisted of matric potential. The existence of tension and the negative pressure was confirmed in elongating tissue (Nonami and Boyer, 1987) and mature tissue (Holbrook et al., 1995; Pockman et al., 1995; Steudle, 1995). Although our approaches were indirect, these crosscheck experiments provide strong evidence for the maintenance of large negative pressures in xylem vessels of transpiring plants. The negative pressure in the apoplast adjacent to elongating cells is a driving force of water movement.
Fig. 5 Relative growth rate plotted against sizes of turgor (A, C, E) and the growth-effective turgor (B, D, F) of tomato leaves grown under 0 kPa VPD (A, B), 1.27 kPa (C, D) and 1.74 kPa (E, F). In A, C, and E, m was considered to be infinitely large. Y in A, C and E is determined from the intercept on the turgor axis at G=0.

for cell elongation.

Boyer and Nonami (1993) analyzed the water mass budget in plants, and concluded that growth flux and transpiration flux are additive. They pointed out that the growth flux might be only 1/100 of the transpiration flux. If significant transpiration takes place, growth-induced water potentials are obscured by transpiration-induced water potentials (Boyer, 1974; Westgate and Boyer, 1984). That was why we measured the water status and transpiration in the dark condition. In such condition, although the transpiration of tomato leaves was relatively low, the transpiration rate linearly increased as the VDP increased (Fig. 3A). Our data indicate that transpiration-induced water potentials by differences in VPD levels significantly affect on the water status and growth of tomato leaves while plants are maintained at optimal hydroponic conditions. When transpiration was significantly large in the dark, the linear relationship between the growth-induced water potential and RGR was parallel translated to the positive direction as the transpiration rate of leaves increased (Fig. 4). Thus, it was found that transpiration flux and growth flux have additive relations. It was considered that the shift in regression lines was caused by transpiration flux. Therefore, it may be concluded that water fluxes for growth and transpiration are different.

We thank T. Kanamoto, M. Kamei, M. Okatani, T. Fukuyama and H. Wada for their assistance for this study.
GROWTH OF TOMATO LEAVES

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