High Plant Density of Cucumber (*Cucumis sativus* L.) Seedlings Mitigates Inhibition of Photosynthesis Resulting from High Vapor-pressure Deficit

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Abstract. To evaluate the effects of plant density on gas exchanges under water stress resulting from high vapor-pressure deficit (VPD), we measured net photosynthetic rate (P_n), transpiration rate, and leaf conductance (g_l) of cucumber (*Cucumis sativus* L.) seedlings before and after raising the VPD at different plant densities. Measurements were conducted continuously using a chamber and weighing method. Five, nine, or 12 seedlings with leaf area index (LAI) of 0.39, 0.73, and 1.10, respectively, were placed in the assimilation chamber. The average VPD in the chamber was raised from 1.1 to 3.7 kPa 30 min after the starting measurement. The P_n and g_l decreased after raising the VPD above the plant community from 1.1 to 3.7 kPa. The VPD near the leaf surface (measured with 3-mm diameter humidity sensors) decreased with increasing LAI of the plant community, whereas average VPD in the whole chamber did not change with LAI. We noted significant negative correlations between the VPD near the leaf surface and P_n and g_l . These results indicate that higher plant density mitigates the inhibition of photosynthesis resulting from high VPD by maintaining a lower VPD near the leaf surface with the development of a thicker boundary layer above the canopy.

In a community with high plant density, as seen in transplant production, the gas exchanges between plant and atmosphere are conducted through a unique boundary layer developed above the canopy (Kim et al., 1996b). The boundary layer varies with changes in the structure of the plant community and microclimatic parameters such as air velocity. The variation of the boundary layer, in turn, affects microclimatic parameters in the plant community such as air temperature, relative humidity, air velocity, and CO₂ concentration (Kim et al., 1996a, 1996b; Kitaya et al., 1998), thereby affecting the gas exchange of the plant community and its consequent growth (Kitaya et al., 2000, 2002, 2004; Shibuya and Kozai, 1998, 2001). Generally, CO₂ supply into the canopy is inhibited with increasing plant density (Shibuya and Kozai, 2001; Yabuki et al., 1967) resulting from the thicker boundary layer that develops above the canopy and the decline of the CO₂ concentration and air velocity in the canopy (Kim et al., 1996a, 1996b; Kitaya et al., 1998). On the other hand, increasing plant density may enhance CO₂ exchange of the leaf when the

CO₂ supply to plants is limited by a decrease in leaf conductance resulting from water stress resulting from high vapor-pressure deficit (VPD). Under higher plant density, the VPD near the leaf surface decreases as a result of the thicker boundary layer (Kim et al., 1996b; Kitaya et al., 1998), which inhibits water vapor exchange between plants and the atmosphere. The gas exchanges at the leaf surface are inhibited by a decrease in leaf conductance with stomatal closing when the VPD near the leaf surface increases (Bunce, 1984; Shibuya et al., 2003, 2006; Woledge et al., 1989). In such a case, high plant density would decrease the VPD near the leaf surface with development of the boundary layer above the plant canopy, thereby likely mitigating the inhibition of CO₂ exchange resulting from the water stress.

In this study, we measured changes in net photosynthetic rate, transpiration rate, and leaf conductance of a seedling community after raising the VPD at various plant densities to evaluate the effects of plant density on gas exchanges under the water stress resulting from high VPD. We developed a new technique to measure leaf conductance continuously based on weight of the plant community and absolute humidity near the leaf surface, which was measured with tiny humidity sensors.

Materials and Methods

Plant material. Cucumber (*Cucumis sativus* L. 'Hokushin') seedlings grown in a

growth chamber for 12 d after seeding were used. The growth conditions were 30/26 °C (light/dark) air temperatures, 50% to 60% relative humidity, and a photosynthetic photon flux (PPF) density of 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ during a photoperiod of 12 h. The seedlings were grown in plastic pots (60-mm diameter, 55-mm high) with vermiculite medium. Nutrient solution (A-type recipe of Otsuka House Solution, diluted by 1/2; Otsuka Chemical Co., Ltd., Osaka, Japan) was supplied from the bottom of the pot as necessary. The seedlings had one foliage leaf (length: 60 to 70 mm) and a pair of cotyledons.

Measuring conditions. Measurements of net photosynthetic rate, transpiration rate, and leaf conductance of the seedlings were conducted in an assimilation chamber [length (L) $\times$ depth (D) $\times$ height (H): 650 $\times$ 400 $\times$ 400 mm] containing a wind tunnel (Fig. 1). Five, nine, or 12 seedlings were placed in the wind tunnel (L $\times$ D $\times$ H: 500 $\times$ 300 $\times$ 300 mm) without intershading between seedlings. The measurements were conducted with two replications for the three plant densities. The different seedlings were used for each measurement. The surface of the growing medium was covered with a plastic film to prevent evaporation. The air velocity windward was controlled at 0.4 $\text{m}\cdot\text{s}^{-1}$ with fans in the wind tunnel. Air temperature and VPD of the assimilation chamber were controlled by introducing controlled air from air conditioning systems connected with the chamber. A blower was used for introducing the controlled air. We used two air conditioning systems to maintain low or high VPD. The assimilation chamber was connected with the low-VPD system at the beginning of the measurement. The VPD in the assimilation chamber was raised by changing the connection to the high-VPD system 30 min after the starting measurement, and then a measurement was conducted for 90 min. The air flow rates of the assimilation chamber were 0.33 and 0.22 $\text{m}^3\cdot\text{min}^{-1}$ under low and high VPD, respectively. Continuous illumination was provided during the measurements with white fluorescent lamps (FPL55EX-N; Matsushita Electric Industrial Co., Ltd., Kadoma, Japan) at a PPF density of 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The CO₂ concentration in the chamber was maintained at $\approx 400 \mu\text{mol}\cdot\text{mol}^{-1}$ without controls.

Measurement method. The weight of the plant community was measured with an electric balance (BW4200; Shimadzu Co., Ltd., Kyoto, Japan) placed below the plant community. The absolute humidity and air temperature near the leaf surface was measured with tiny humidity sensors (RHM-1000S; Ricoh Elemex Co., Ltd., Nagoya, Japan). The probes (3-mm diameter, 20-mm long) of the sensor were placed 3 mm above and below the center of the foliage leaf of the central seedling of the community (Fig. 1). Leaf temperature was measured by contacting a junction of a thermocouple (T-type, 0.1-mm diameter) on the abaxial leaf surface. The vapor pressure near the leaf surface was estimated from the absolute humidity. The VPD near the leaf surface was estimated by

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Fig. 1. Schematic diagram of the experimental assimilation chamber with wind tunnel: (A) horizontal section; (B) cross-section.

subtracting the vapor pressure near the leaf surface from saturating vapor pressure at the leaf temperature. The CO_2 concentration at the inlet and outlet of the assimilation chamber was sampled alternately at 1-min intervals with an air pump at a flow rate of $1.0 \text{ L} \cdot \text{min}^{-1}$; samples were measured with an infrared gas analyzer (LI-7000; LI-COR Inc., Lincoln, NE). Effects of air leak of the chamber were neglected, because air pressure in the chamber was maintained positive by introducing air with the blower.

The average air temperature and relative humidity in the assimilation chamber were measured with a temperature and humidity recorder (TR-72s; T&D Co. Ltd., Matsumoto, Japan) placed leeward of the wind tunnel. The average VPD in the assimilation chamber was estimated based on air temperature and relative humidity. The air velocity at 5 mm above the leaf surface under each plant density condition was measured with a hot-wire anemometer (Anemomaster Model 6071; Kano-max Japan, Inc., Suita, Japan) at the end of each set of measurements.

Leaf area index (LAI) of the plant community was estimated based on the canopy floor area and the total leaf area measured at the end of each experiment. The average LAI values at

plant densities of five, nine, and 12 plants per chamber (low, middle, and high plant density) were 0.39, 0.73, and 1.10, respectively.

Calculations. The transpiration rate per leaf area of the plant community (Tr , $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \text{ H}_2\text{O}$) was estimated based on the change in weight of the plant community with the equation:

$$Tr = \frac{W_1 - W_2}{t_2 - t_1} \cdot \frac{1}{m_v} \cdot \frac{1}{A}$$

where W_1 and W_2 (g) were the weights of the plant community at times t_1 and t_2 (s), respectively, m_v was the molecular weight of water ($18 \text{ g} \cdot \text{mol}^{-1} \text{ H}_2\text{O}$), and A was total leaf area of the plant community (m^2).

The total vapor diffusion resistance (R_{l+b} , $\text{s} \cdot \text{m}^{-1}$) from inside the leaf to the atmosphere, accounting for the vapor diffusion resistance of leaf (R_l , $\text{s} \cdot \text{m}^{-1}$) and leaf boundary layer resistance from leaf surface to (R_b , $\text{s} \cdot \text{m}^{-1}$), was estimated based on the diffusion vapor model (Monteith and Unsworth, 2008) using the equation:

$$R_{l+b} = R_l + R_b = \frac{y_1 - y_a}{m_v \cdot 0.5Tr}$$

where y_a and y_1 ($\text{g} \cdot \text{m}^{-3} \text{ H}_2\text{O}$) were the absolute humidity near the leaf surface and inside the

leaf, respectively, and $0.5Tr$ ($\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \text{ H}_2\text{O}$) the transpiration rate per total leaf surface area. y_a was determined based on the average of the absolute humidity above and below the leaf surface. y_1 was assumed to be the saturated absolute humidity at the leaf temperature, because the intercellular space in the leaf was assumed to be saturated with water vapor. In this study, y_a and y_1 measured at the center of the foliage leaves were taken with two to three replications as the representative values.

The R_b value was determined using a model plant community. The leaf of the model plant was made with a regular pentagon-shaped piece of wet paper (60 mm long; Toyo Roshi Kaisha, Ltd., Tokyo, Japan). Transpiration rate (Tr_m , $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \text{ H}_2\text{O}$) of the model plant community, absolute humidity (y_{as} , $\text{g} \cdot \text{m}^{-3} \text{ H}_2\text{O}$) near the model leaf surface, absolute humidity (y_{ml} , $\text{g} \cdot \text{m}^{-3} \text{ H}_2\text{O}$) inside the model leaf, and leaf temperatures were measured using the same methods as in the cucumber plant community. The R_b was estimated with the equation:

$$R_b = \frac{y_m - y_a}{m_v \cdot 0.5Tr_m}$$

The leaf conductance (g_l , $\text{m} \cdot \text{s}^{-1}$) was estimated with the equation:

$$g_l = \frac{1}{R_l} = \frac{1}{R_{l+b} - R_b}$$

The photosynthetic rate (P_n , $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \text{ CO}_2$) of the plant community per leaf area was estimated with the equation:

$$P_n = v \cdot \frac{C_{in} - C_{out}}{A}$$

where C_{in} and C_{out} ($\text{mol} \cdot \text{m}^{-3} \text{ CO}_2$) was the CO_2 concentration at the inlet and outlet of the assimilation chamber, respectively, and v ($\text{m}^3 \cdot \text{s}^{-1}$) was the air flow rate of the chamber.

Statistics. Relationships between the variables were analyzed by Pearson's product-moment correlation coefficient at $P = 0.10$, 0.05, and 0.01.

Results and Discussion

The air velocity at 5 mm above the leaf surface decreased with increasing plant density with values of 0.27, 0.17, and $0.10 \text{ m} \cdot \text{s}^{-1}$, respectively, for low, middle, and high plant density. The R_b determined by using wet mode leaf was 7.3×10^{-2} , 8.5×10^{-2} , and $9.5 \times 10^{-2} \text{ s} \cdot \text{m}^{-1}$ for low, middle, and high plant density, respectively. The R_b tended to be decreased linearly with the increasing air velocity. The relationship between the air velocity and the R_b is similar to previous studies (Yabuki, 2004). This lower air velocity and R_b with higher plant density was caused by the development of a thicker boundary layer above the canopy. The time courses of average air temperature, relative humidity, and VPD in the assimilation chamber are shown in Figure 2. The air temperature was maintained at 30°C . The relative humidity was maintained at 73% before raising the VPD and then decreased to 13% afterward. The VPD was maintained at 1.1 kPa and then increased to 3.7 kPa. These values were nearly the same at all plant densities. The

Fig. 2. Time courses of average air temperature, relative humidity, and vapor-pressure deficit (VPD) in the chamber before and after raising the VPD. Examples of the measurements at low plant density are shown.

average VPD values near the leaf surface before raising the VPD were 0.32, 0.29, and 0.29 kPa at low, middle, and high plant density, respectively (Fig. 3). The higher VPD at lower plant density before raising the VPD was probably the result of a thinner boundary layer above the canopy. At 60 to 90 min after raising the VPD, the VPD near the leaf surface tended to decrease with increasing plant density with values of 1.96, 1.52, and 1.08 kPa, respectively, at low, middle, and high plant density (Fig. 3). The lower VPD near leaf surface of higher plant density was probably the result of a thicker boundary layer developing above the canopy, which would inhibit vapor diffusion. After raising the VPD, near the leaf surface, the VPD decreased with LAI ($P < 0.05$; Fig. 4), whereas before the VPD was raised, there was no significant correlation with LAI.

The Tr increased for 10 min after raising the VPD and then decreased (Fig. 3). At 0 to 30 min after raising the VPD, the Tr decreased with increasing plant density with average values of 5.96, 4.86, and 4.52 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ H_2O , respectively, at low, middle, and high plant density. There was a significant negative correlation between LAI and Tr 0 to 30 min after raising the VPD, indicating that water loss from the plant immediately after the VPD rise was greater at lower plant density. There was no significant correlation between LAI and Tr 60 to 90 min after raising the VPD (Fig. 5).

The g_1 decreased after the VPD was raised (Fig. 3). At 60 to 90 min after raising the

Fig. 3. Time courses of vapor-pressure deficit (VPD) near the leaf surface, transpiration rate (Tr), leaf conductance (g_1), and net photosynthetic rate (P_n) of cucumber seedlings at different plant densities before and after raising the VPD. Averages of two replications are shown. Missing values in net photosynthetic rate are caused by disturbances of CO_2 concentration.

VPD, the g_1 tended to increase with increasing plant density with average values of 3.5, 4.2, and 5.9 $\text{mm}\cdot\text{s}^{-1}$, respectively, at low, middle, and high plant density. The start of decrease in g_1 tended to be delayed for 10 min after raising the VPD under high plant density. The decrease in g_1 after the VPD rise was probably the result of stomatal closing caused by excessive transpiration at the high VPD, which was observed in a previous study (Shibuya et al., 2006). After raising the VPD, the g_1 increased with LAI ($P < 0.10$; Fig. 5), whereas before the VPD was raised, there was no significant correlation with LAI.

The P_n decreased after raising the VPD (Fig. 3). At 60 to 90 min after the VPD rise, the P_n increased with plant density with average values of 13.0, 14.2, and 15.5 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ CO_2 , respectively, at low, middle, and high plant density. The decrease in P_n after raising the VPD was probably the result of the decrease in g_1 . After raising the VPD, the P_n increased significantly with increasing LAI ($P < 0.01$; Fig. 5), whereas before the VPD was raised, there was no significant correlation with LAI. The increase in thickness of boundary layer with LAI probably did not affect direct CO_2 assimilation of the seedlings in this experiment, because P_n before raising VPD was not changed with LAI, which affected development of the boundary layer. This result indicates that CO_2 concentration near the leaf

Fig. 4. Effects of leaf area index (LAI) on vapor-pressure deficit (VPD) near the leaf surface of cucumber seedlings at low (average 0 to 30 min before raising VPD, open circles) and high VPD (average 60 to 90 min after raising VPD, closed circles).

surface was probably unaffected significantly by the thickness of the boundary layer as the VPD near the leaf surface.

There was no significant correlation between VPD near the leaf surface and Tr (Fig. 6). With increasing plant density, the g_1 varied inversely with VPD near the leaf surface; therefore, Tr (i.e., the product of g_1 and VPD) probably did not change significantly. In other words, the direct effect of

Fig. 5. Effects of leaf area index (LAI) on transpiration rate (T_r), leaf conductance (g_l), and net photosynthetic rate (P_n) of cucumber seedlings at high vapor-pressure deficit (average 60 to 90 min after raising VPD).

VPD on T_r and the indirect effect of VPD resulting from the change in g_l compensated for each other in this study. In contrast, P_n and g_l decreased with increasing VPD near the leaf surface (Fig. 6). There were significant negative correlations between VPD near the leaf surface and P_n and g_l (both $P < 0.01$). These results indicate that a higher plant density maintains a lower VPD near the leaf surface with development of the boundary layer above the canopy, which mitigates the decrease in g_l after stomatal closure resulting from the excessive transpiration immediately after raising the VPD, thereby mitigating the inhibition of P_n . The LAI of 0.39 to 1.10 examined in this experiment is

Fig. 6. Effects of vapor-pressure deficit (VPD) near the leaf surface on leaf conductance (g_l) and net photosynthetic rate (P_n) of cucumber seedlings at high VPD (average 60 to 90 min after raising VPD).

relatively low compared with the plant community in commercial production. If measuring was conducted for much higher LAI, P_n per leaf area would be decreased by inter-shading and inhabitation of gas exchange by developing a boundary layer. Mitigating the inhibition of P_n under high plant density probably occurs in only cases in which stomatal closure resulting from high VPD affects a limiting factor of photosynthesis before other factors.

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