BRIEF COMMUNICATION

Stomatal and non-stomatal limitations to photosynthesis in field-grown grapevine cultivars

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Abstract

Diurnal changes of photosynthesis in the leaves of grapevine (Vitis vinifera × V. labrusca) cultivars Campbell Early and Kyoho grown in the field were compared with respect to gas exchanges and actual quantum yield of photosystem 2 (ΦPS2) in late May. Net photosynthetic rate (PN) of the two cultivars rapidly increased in the morning, saturated at photosynthetic photon flux density (PPFD) from 1200 to 1500 μmol m⁻² s⁻¹ between 10:00 and 12:00 and slowly decreased after midday. Maximum PN was 13.7 and 12.5 μmol m⁻² s⁻¹ in Campbell Early and Kyoho, respectively. The stomatal conductance (gs) and transpiration rate changed in parallel with PN, indicating that PN was greatly affected by gs. However, the decrease in PN after midday under saturating PPFD was also associated with the observed depression of ΦPS2 at high PPFD. The substantial increase in the leaf to air vapour pressure deficit after midday might also contribute to decline of gs and PN.

Additional key words: gas exchange, quantum yield, stomatal conductance, transpiration rate, vapour pressure deficit.

Plants are exposed to variable photosynthetic photon flux density (PPFD), temperature and humidity in the field. At low PPFD, more than 80 % of the absorbed light energy may be utilized for CO₂ assimilation. However, excess light during midday can inhibit photosynthesis (Chaumont et al. 1994, Bertamini and Nedunchezhian 2004, Yu and Lee 2004).

The midday depression of photosynthesis likely results primarily from long periods of high PPFD (Chaves et al. 1987, Correia et al. 1990). Other possible causes of midday depression include an increase in leaf to air vapour pressure deficit (VPD) (Pathre et al. 1998), high temperature (Singh et al. 1996) and feedback inhibition of photosynthesis by sugar accumulation (Chaumont et al. 1994).

The depression might be due to stomatal and non-stomatal limitations. The stomatal closure during midday can decrease the local intercellular CO₂ concentration (ci), subsequently inhibiting photosynthesis. In several cases, however, the apparent carboxylation efficiency decreased during midday depression, while ci remained constant (Correia et al. 1990, Sinha et al. 1997). The decrease in carboxylation efficiency, causing non-stomatal inhibition of photosynthesis, might be due to photodamage. The photodamage commonly occurs when absorbed light exceeds the amount required for electron transport and CO₂ assimilation (Müller et al. 2001). High PPFD, combined with environmental stress, such as high temperature or drought, intensifies photodamage of assimilatory apparatus, thereby inhibiting photosynthesis (Gamon and Pearcy 1990, Ohashi et al. 2006).

In the present study, diurnal changes and midday depression of photosynthesis in grapevine cultivars grown in the field were compared on a clear day with...
respect to gas exchange and actual quantum yield of photosystem 2 ($\Phi_{PS2}$) to provide practical information regarding photosynthetic characteristics of the two cultivars. Furthermore, effects of environmental factors, such as high PPFD, high temperature and high VPD were discussed.

Two-year-old grapevine ($Vitis vinifera \times V. labrusca$) Campbell Early and Kyoho cultivars were grown in 6-dm$^3$ pots containing soil:peat:sand (1:2:1, v/v/v) in the field. The plants were irrigated daily and supplied with half-strength Hoagland's solution (Hoagland and Arnon 1950) biweekly. Diurnal changes of gas exchange and chlorophyll fluorescence were measured on the south-facing leaves of each grapevine, near the end of May in Suwon, Korea. The measurements were made at least five times, but the representative results were presented in this paper. The same leaf was analyzed for each sampling throughout the day. Each leaf was fully exposed and oriented to sunlight during measurements for being absorbed the highest possible PPFD.

Gas exchange rates were measured using a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) equipped with an infrared gas analyzer. The leaf area clipped by a clear top chamber was 6 cm$^2$. Net photosynthetic rate ($P_N$), stomatal conductance ($g_s$), transpiration rate ($E$) and leaf to air VPD based on $T_{leaf}$ ($VpdL$) were determined using simultaneous measurements of $CO_2$ and $H_2O$ vapour flux, air temperature and leaf temperature ($T_{leaf}$). Incident PPFD on the leaf surface and $T_{leaf}$ were measured using a chamber-in quantum sensor and a thermocouple, respectively. Each measurement was performed each hour during the day, within 3 min of closing the leaf chambers.

Diurnal time courses of $\Phi_{PS2}$ were measured with a

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**Fig. 1.** Diurnal changes of PPFD (A), leaf temperature, $T_{leaf}$ (B), stomatal conductance, $g_s$ (C), transpiration rate, $E$ (D), net photosynthetic rate, $P_N$ (E), water use efficiency, $P_N/E$ (F), $\Phi_{PS2}$ (G) and leaf to air vapour pressure deficit based on $T_{leaf}$, $VpdL$ (H) in the leaves of grapevine cultivars Campbell Early (closed circles) and Kyoho (open circles), measured in the field in late May. Vertical bars represent SE of the means ($n = 5$).
portable pulse amplitude modulation fluorometer (PAM-2000, Walz, Effeltrich, Germany). The leaf was carefully clamped with a leaf-clip holder. When the steady state fluorescence (F_s) value was stabilized, maximum fluorescence (F_m) was obtained by applying a saturation pulse of approximately 7000 μmol m⁻² s⁻¹ PPFD for 0.8 s in order to achieve a complete saturation of photosystem 2 (PS 2) reaction centers. \( \Phi_{PS2} \), described as the fraction of absorbed light utilized through photochemistry, was estimated using the equation

\[
\Phi_{PS2} = \frac{(F_m' - F_s)}{F_m} 
\]


Changes in PPFD and \( T_{leaf} \), as measured during the day, were found to be consistent with expected values. Maximum PPFD was measured between 11:00 and 13:00 with a resulting value of approximately 1500 μmol m⁻² s⁻¹ (Fig. 1A). Maximum \( T_{leaf} \) measured at 13:00 was about 31 °C (Fig. 1B).

Significant diurnal changes in \( g_s \), \( E \), \( P_N \), \( \Phi_{PS2} \) and VpdL were observed in Campbell Early and Kyoho cultivars grown in the field. \( g_s \) rapidly increased in the morning and decreased slowly after midday (Fig. 1C). Between 09:00 and 16:00, Campbell Early exhibited higher values of \( g_s \) than Kyoho. Maximum values of \( g_s \) between 10:00 and 12:00 were 0.21 and 0.15 mol m⁻² s⁻¹ in Campbell Early and Kyoho, respectively. Contrary to the previous report, which stated that maximum stomatal opening of an individual leaf was recorded at a PPFD of 130 to 300 μmol m⁻² s⁻¹ (Kriedemann and Smart 1971), maximum \( g_s \) of Campbell Early and Kyoho grapevine cultivars grown in the field were measured at about 1000 μmol m⁻² s⁻¹.

The diurnal courses of \( E \) and \( P_N \) changed parallelly to \( g_s \), consistent with the previous reports for grapevines (Chaves et al. 1987, Flexas et al. 1999). \( E \) and \( P_N \) also rapidly increased in the morning and slowly decreased after midday (Fig. 1D,E). Between 10:00 and 16:00, \( E \) and \( P_N \) were higher in Campbell Early than in Kyoho. Maximum \( P_N \) measured from 11:00 to 12:00 was 13.7 and 12.5 μmol m⁻² s⁻¹ in Campbell Early and Kyoho, respectively, indicating that \( E \) and \( P_N \) were greatly affected by \( g_s \) in both cultivars. \( E \) was linearly related to \( g_s \) in Campbell Early \( (r^2 = 0.84**) \) and Kyoho \( (r^2 = 0.62**) \) (Fig. 2A). Also, \( P_N \) was curvilinearly related to \( g_s \) in Campbell Early \( (r^2 = 0.59*) \) and Kyoho \( (r^2 = 0.91**) \) (Fig. 2B). Compared to the level of decline in \( P_N \), the reduction of \( E \) was more apparent in Kyoho than in Campbell Early, indicating that water use efficiency was higher in Kyoho at midday. After midday, \( P_N/E \) values were higher in Kyoho than in Campbell Early (Fig. 1F).

The non-linear relationship between \( P_N \) and \( g_s \) in the two cultivars was likely caused by the greater depression of \( P_N \) during midday, compared to the level of stomatal closure. Although stomata impose a large limitation on \( CO_2 \) assimilation (Jones 1985), non-stomatal factors also cause the midday depression of \( P_N \). For example, Correia et al. (1990) reported that carboxylation efficiency in \( V. vinifera \) during midday decreased, concurrent with a pronounced depression in \( CO_2 \) assimilation and stomatal conductance.

High PPFD might cause a non-stomatal limitation to photosynthesis in Campbell Early and Kyoho as \( P_N \) depressed regardless of \( T_{leaf} \) (Chaves et al. 1987, Correia et al. 1990). \( P_N \) depression occurs when more light energy than is required for \( CO_2 \) assimilation is absorbed (Correia et al. 1990, Sinha et al. 1997, Müller et al. 2001, Bertamini and Neduchezhian 2004).

Under high PPFD, Kyoho might be more sensitive to photoinhibition than Campbell Early, since the slope of the decrease in \( P_N \) after midday was greater in Kyoho than in Campbell Early at the same level of PPFD (Fig. 1E). Also, the \( P_N \) depression occurred earlier in Kyoho than in Campbell Early. However, the midday depression in Kyoho was not as severe as observed by Flexas et al. (1999), who evaluated the level of photosynthesis in relation to water stress in young potted grapevines. \( P_N \) of Kyoho would be expected to reach high levels earlier in the morning and midday depression would be more severe during summer months or during periods of high environmental stress.

Effects of high PPFD were further studied by measuring diurnal changes in overall quantum yield of PS 2, using a portable fluorometer. The values of \( \Phi_{PS2} \) changed significantly during the day, closely following changes in PPFD, with minimum values around 0.2 (Fig. 1G). \( \Phi_{PS2} \) values were highest in the morning, and dropped when PPFD increased between 10:00 and 13:00, then increased again when PPFD decreased. At the end of the day, \( \Phi_{PS2} \) values of the two cultivars were similar to
those at the beginning of the day. The reversible decline observed in ΦPS2 is possibly an expression of the onset of protective mechanisms which allow for an improved radiationless de-excitation of PS 2 (Adams III et al. 1999). These mechanisms thus cause midday depression at high PPFD, limiting further damage of photosynthetic apparatus. Radiationless de-excitation related to zeaxanthin formation via xanthophyll cycle (Demmig-Adams et al. 1996, Müller et al. 2001, Čaňová et al. 2008) might also lower the light energy used in photochemistry.

\[ P_N = \text{measured at field conditions with plotted with incident PPFD (Fig. 3A). The light response curves were similar to those derived from laboratory measurements (Yu and Lee 2004). In the field, however, } P_N \text{ of the two cultivars was saturated at a higher PPFD than in the laboratory: 1200 to 1500 \text{ versus } 300 \text{ to } 600 \text{ μmol m}^{-2} \text{s}^{-1} (Downton et al. 1987, Yu and Lee 2004). These results indicate that photosynthetic capacity was affected by the surrounding environment of the tested leaf. For example, light saturation for individual leaves varied according to their growing conditions (Kriedemann and Smart 1971, Liao et al. 2006).}

PPFD may not be solely responsible for the reduction in \( P_N \) under field conditions. Stomata respond dynamically to VpdL, presumably because of the effect of VpdL on the demand for water flowing through the epidermis and the stomatal complex. Thus, \( P_N \) and \( g_s \) may be affected by VpdL at midday. Pathre et al. (1998) reported that, in experiments in which leaves were exposed to saturating PPFD (800 μmol m\(^{-2}\) s\(^{-1}\)) and a gradual stepwise increase in VpdL occurred, a marked depression in \( P_N \) was obtained. Similarly, the decline in \( P_N \) and \( g_s \) in Campbell Early and Kyoho might be associated with high PPFD (> 1000 μmol m\(^{-2}\) s\(^{-1}\)) and a substantial increase in VpdL (> 1.5 kPa). \( P_N \) and \( g_s \) increased until 11:00 and then decreased gradually. In contrast, VpdL increased continuously to the maximum values of 2.5 and 3.1 kPa, between 13:00 and 14:00 in Campbell Early and Kyoho, respectively (Fig. 1H). According to the results, the increase in VpdL further reduced \( P_N \). Although a high VpdL may be the dominant factor for reducing \( P_N \) (Pathre et al. 1998), light contributes to changes in the photochemical status of the leaf under varying environmental conditions.

High temperature (Singh et al. 1996) and feedback inhibition of photosynthesis by sugar accumulation (Sinha et al. 1997) have been suggested as possible causes of midday decline of \( P_N \). Photorespiration is another potential non-stomatal factor inhibiting photosynthesis (Kozaki and Takeba 1996). The relationship between light and dark reactions is not straightforward, since ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) uses the reductants NADPH and ATP for both carboxylation and oxygenation (Rosenqvist and Van Kooten 2003). This implies that some of the reductants are used to oxidize reduced carbon and thus consume \( O_2 \) producing \( CO_2 \). Although the specificity of Rubisco for \( CO_2 \) is about 1000 times higher under ambient conditions, under certain conditions, Rubisco can contribute to a substantial rate of photorespiration (Sharkey 1988).

In order to analyze the potential effects of \( T_{leaf} \) on the diurnal pattern, \( P_N \) was plotted as a function of \( T_{leaf} \). At a given temperature and PPFD > 1200 μmol m\(^{-2}\) s\(^{-1}\), \( P_N \) measured in the afternoon was consistently lower than that measured in the morning (Fig. 3B). The declines in \( P_N \) observed in the two cultivars in the afternoon did not appear to be a simple response to \( T_{leaf} \). The temperature response curve for diurnal changes in \( P_N \) suggested that temperature variation below 31 °C does not have a direct effect on the afternoon decline of \( P_N \) in Campbell Early and Kyoho.

In conclusion, the reduction of \( P_N \) in Campbell Early and Kyoho was found to be caused by the increase in VpdL and high PPFD. However, these results do not clearly explain the mechanisms causing the difference in the degree of midday depression in \( P_N \) between the two cultivars. Thus, various approaches, including the evaluation of radiationless energy dissipation levels and the antioxidative system against active oxygen species induced at high PPFD, are needed.

Fig. 3. A - net photosynthetic rate (\( P_N \)) in response to PPFD measured in grapevine cultivars Campbell Early (closed circles) and Kyoho (open circles) in late May. Vertical bars represent SE of the means (n = 5); B - effect of leaf temperature (\( T_{leaf} \)) on diurnal changes of \( P_N \) measured in the morning (circles) and afternoon (triangles) in late May.
References


