BRIEF COMMUNICATION

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

D.J. YU*, S.J. KIM* and H.J. LEE**[1]

*Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea**
*Department of Plant Science, Seoul National University, Seoul 151-921, Korea***

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 (Φ_{PS2}) in late May. Net photosynthetic rate (P_N) 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 P_N was 13.7 and 12.5 μmol m^{-2} s^{-1} in Campbell Early and Kyoho, respectively. The stomatal conductance (g_s) and transpiration rate changed in parallel with P_N, indicating that P_N was greatly affected by g_s. However, the decrease in P_N after midday under saturating PPFD was also associated with the observed depression of Φ_{PS2} at high PPFD. The substantial increase in the leaf to air vapour pressure deficit after midday might also contribute to decline of g_s and P_N.

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 CO_2 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 CO_2 concentration (c_i), subsequently inhibiting photosynthesis. In several cases, however, the apparent carboxylation efficiency decreased during midday depression, while c_i 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 CO_2 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

---

Received 16 February 2007, *accepted* 8 August 2007.

Abbreviations: c_i - intercellular CO_2 concentration; E - transpiration rate; F_m' - maximum fluorescence in light-adapted state; Φ_{PS2} - actual quantum yield of photosystem 2; F_s - steady state fluorescence; g_s - stomatal conductance; P_N - net photosynthetic rate; PPFD - photosynthetic photon flux density; PS 2 - photosystem 2; Rubisco - ribulose-1,5-bisphosphate carboxylase/oxygenase; T_{leaf} - leaf temperature; VPD - vapour pressure deficit; VpdL - leaf to air vapour pressure deficit based on T_{leaf}.

Acknowledgements: This work was supported by Grape Research Center funded by Agricultural R&D Promotion Center, Seoul, Korea. We thank National Instrumentation Center for Environmental Managements, Seoul National University, Korea for the use of photosynthesis systems.

[1] Corresponding author; fax: (+82) 2 873 2056, e-mail: heejlee@snu.ac.kr

respect to gas exchange and actual quantum yield of photosystem 2 (Φ_{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* × *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 Φ_{PS2} were measured with a

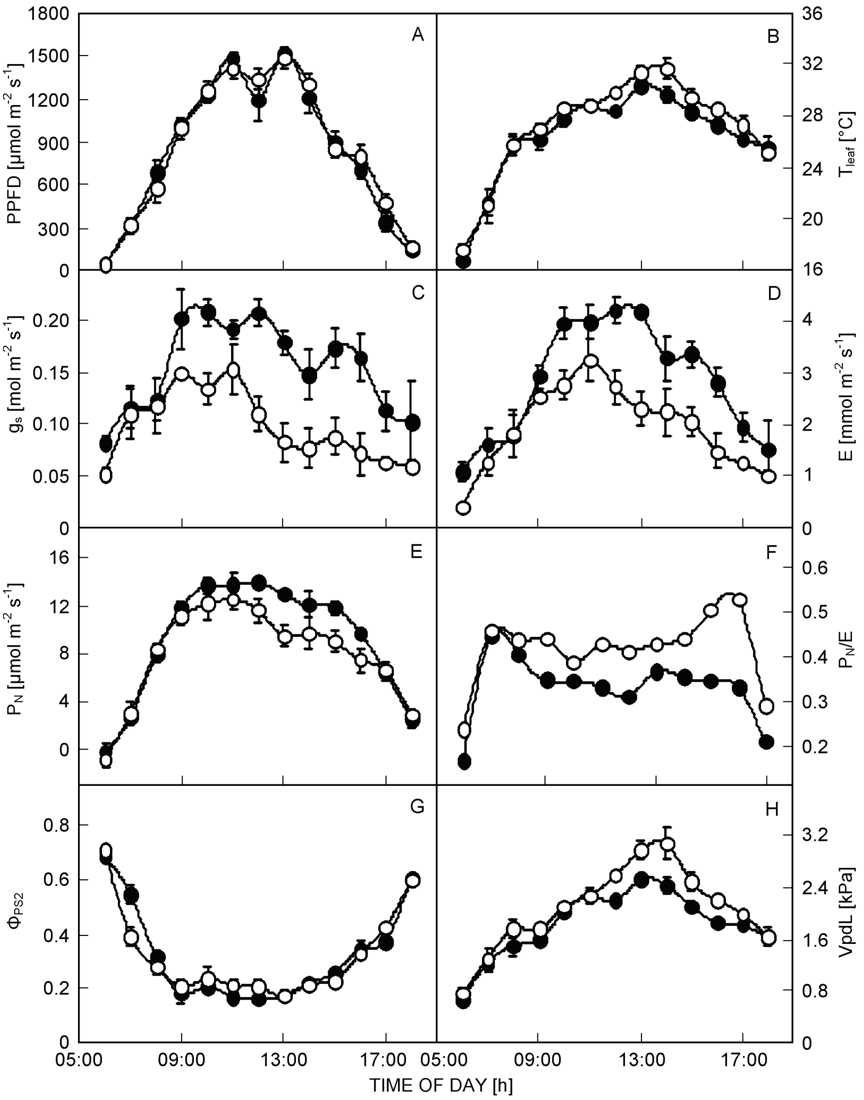

Fig. 1. Diurnal changes of PPFD (*A*), leaf temperature, T_{leaf} (*B*), stomatal conductance, g_s (*C*), transpiration rate, E (*D*), net photosynthetic rate, P_N (*E*), water use efficiency, P_N/E (*F*), Φ_{PS2} (*G*) and leaf to air vapour pressure deficit based on T_{leaf} , VpdL (*H*) in the leaves 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. Φ_{PS2}, described as the fraction of absorbed light utilized through photochemistry, was estimated using the equation $\Phi_{PS2} = (F_m' - F_s)/F_m'$ (Demmig-Adams *et al.* 1996, Adams III *et al.* 1999).

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. 1*A*). Maximum T_{leaf} measured at 13:00 was about 31 °C (Fig. 1*B*).

Significant diurnal changes in g_s, E, P_N, Φ_{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. 1*C*). 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. 1*D*,*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. 2*A*). Also, P_N was curvilinearly related to g_s in Campbell Early (r^2 = 0.59*) and Kyoho (r^2 = 0.91**) (Fig. 2*B*). 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. 1*F*).

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 Nedunchezhian 2004).

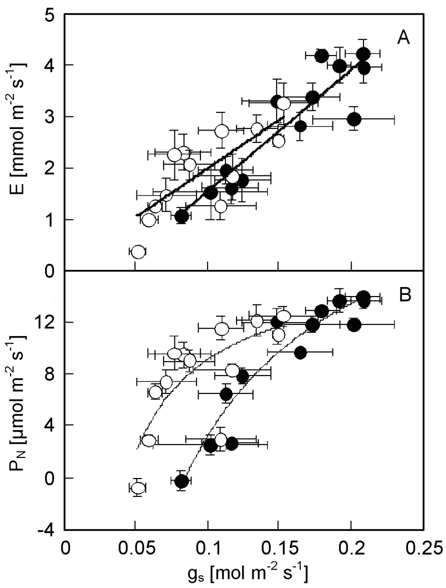

Fig. 2. Relationship between stomatal conductance (g_s) and transpiration rate (E) (*A*) or net photosynthetic rate (P_N) (*B*) in the leaves of Campbell Early (*closed circles*) and Kyoho (*open circles*) grapevine cultivars, measured in the field in late May. *Horizontal* or *vertical bars* represent SE of the means (*n* = 5).

Under high PPFD, Kyoho might be more sensitive to photoinhibition than Campbell Early, since the slope of the decline in P_N after midday was greater in Kyoho than in Campbell Early at the same level of PPFD (Fig. 1*E*). 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 Φ_{PS2} changed significantly during the day, closely following changes in PPFD, with minimum values around 0.2 (Fig. 1*G*). Φ_{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, Φ_{PS2} values of the two cultivars were similar to those at the beginning of the day. The reversible decline observed in Φ_{PS2} 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.

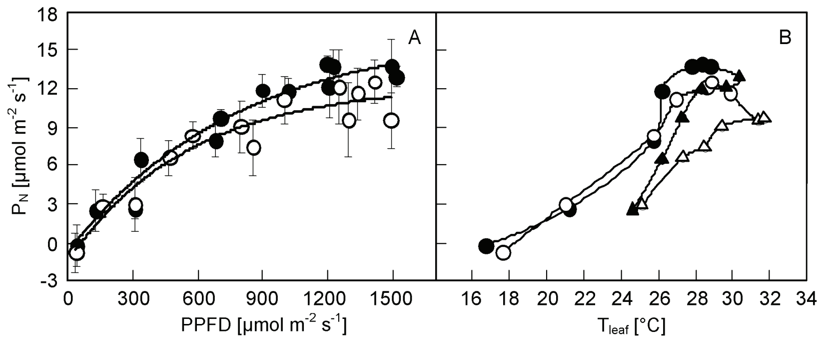

Fig. 3. *A* - net photosynthetic rate (P_N) in response to PPFD measured in grapevine cultivars Campbell Early (*closed circles*) and Kyoho (*open circles*) in late May. *Vertical bars* represent SE of the means (*n* = 5); *B* - effect of leaf temperature (T_{leaf}) on diurnal changes of P_N measured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

P_N measured at field conditions was plotted with incident PPFD (Fig. 3*A*). The light response curves were similar to those derived from laboratory measurements (Yu and Lee 2004). In the field, however, P_N of the two cultivars was saturated at a higher PPFD than in the laboratory: 1200 to 1500 *versus* 300 to 600 µmol m^{-2} 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. 1*H*). 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. 3*B*). 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.

References

Adams III, W.W., Demmig-Adams, B., Logan, B.A., Barker, D.H., Osmond, C.B.: Rapid changes in xanthophyll cycle-dependent energy dissipation and photosystem II efficiency in two vines, *Stephania japonica* and *Smilax australis*, growing in the understory of an open eucalyptus forest. - Plant Cell Environ. **22**: 125-136, 1999.

Bertamini, M., Nedunchezhian, N.: Photoinhibition and recovery of photosystem 2 in grapevine (*Vitis vinifera* L.) leaves grown under field conditions. - Photosynthetica **41**: 611-617, 2004.

Čaňová, I., Ďurkovič, J., Hladká, D.: Stomatal and chlorophyll fluorescence characteristics in European beech cultivars during leaf development. - Biol. Plant. **52**: 577-581, 2008.

Chaumont, M., Morot-Gaudry, J.F., Foyer, C.H.: Seasonal and diurnal changes in photosynthesis and carbon partitioning in *Vitis vinifera* leaves in vines with and without fruit. - J. exp. Bot. **45**: 1235-1243, 1994.

Chaves, M.M., Harley, P.C., Tenhunen, J.D., Lange, O.L.: Gas exchange studies in two Portuguese grapevines. - Physiol. Plant. **70**: 639-647, 1987.

Correia, M.J., Chaves, M.M.C., Pereira, J.S.: Afternoon depression in photosynthesis in grapevine leaves: evidence for a high light stress effect. - J. exp. Bot. **41**: 417-426, 1990.

Demmig-Adams, B., Adams III, W.W., Barker, D.H., Logan, B.A., Bowling, D.R., Verhoeven, A.S.: Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to radiationless dissipation of excess excitation. - Physiol. Plant. **98**: 253-264, 1996.

Downton, W.J.S., Grant, W.J.R., Loveys, B.R.: Diurnal changes in the photosynthesis of the field-grown grapevines. - New Phytol. **105**: 71-80, 1987.

Flexas, J., Escalona, J.M., Medrano, H.: Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. - Plant Cell Environ. **22**: 39-48, 1999.

Gamon, J.A., Pearcy, R.W.: Photoinhibition in *Vitis vinifera*: interactive effects of sunlight, temperature and water status. - Plant Cell Environ. **13**: 267-275, 1990.

Hoagland, D.R., Arnon, D.I.: The water-culture method of growing plants without soil. - Coll. agr. exp. Sta. Circular **347**: 1-32, 1950.

Jones, H.G.: Partitioning stomatal and non-stomatal limitation to photosynthesis. - Plant Cell Environ. **8**: 95-104, 1985.

Kozaki, A., Takeba, G.: Photorespiration protects C_3 plants from photooxidation. - Nature **384**: 557-560, 1996.

Kriedemann, P.E., Smart, R.E.: Effects of irradiance, temperature and leaf water potential on photosynthesis of vine leaves. - Photosynthetica **5**: 6-15, 1971.

Liao, J.-X., Ge, Y., Guan, B.-H., Jiang, Y.-P., Chang, J.: Photosynthetic characteristics and growth of *Mosla hangchowensis* and *M. dianthera* under different irradiances. - Biol. Plant. **50**: 737-740, 2006.

Müller, P., Li, X.P., Niyogi, K.K.: Non-photochemical quenching: a response to excess light energy. - Plant Physiol. **125**: 1558-1566, 2001.

Ohashi, Y., Nakayama, N., Saneoka, H., Fujita, K.: Effects of drought stress on photosynthetic gas exchange, chlorophyll fluorescence and stem diameter of soybean plants. - Biol. Plant. **50**: 138-141, 2006.

Pathre, U., Sinha, A.K., Shirke, P.A., Sane, P.V.: Factors determining the midday depression of photosynthesis in trees under monsoon climate. - Trees **12**: 472-481, 1998.

Rosenqvist, E., Van Kooten, O.: Chlorophyll fluorescence: a general description and nomenclature. - In: De Ell, J.R., Toivonen, P.M.A. (ed.): Practical Application of Chlorophyll Fluorescence in Plant Biology. Pp. 31-77. Kluwer Academic Publishers, Dordrecht - London 2003.

Sharkey, T.D.: Estimating the rate of photorespiration in leaves. - Physiol. Plant. **73**: 47-52, 1988.

Singh, M., Chaturvedi, R., Sane, P.V.: Diurnal and seasonal photosynthetic characteristics of *Populus deltoides* Marsh. leaves. - Photosynthetica **32**: 11-21, 1996.

Sinha, A.K., Shirke, P.A., Pathre, U., Sane, P.V.: Midday depression in photosynthesis: effect on sucrose-phosphate synthase and ribulose-1,5-bisphosphate carboxylase in leaves of *Prosopis juliflora* (Swartz) DC. - Photosynthetica **34**: 115-124, 1997.

Yu, D.J., Lee, H.J.: CO_2 assimilation and photosystem II activities in Campbell Early and Kyoho grapevine cultivars at various photosynthetic photon flux densities. - J. korean Soc. hort. Sci. **45**: 83-87, 2004.