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Diurnal variation of gas exchange, chlorophyll fluorescence, and xanthophyll cycle components of maize hybrids released in different years

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Abstract

Diurnal variation of gas exchange, chlorophyll (Chl) fluorescence, and xanthophyll cycle components of three maize (*Zea mays* L.) hybrids released in different years, *i.e.* Baimaya (1950s), Zhongdan2 (1970s), and Nongda108 (1990s), were compared. On cloudless days, the newer hybrids always had higher net photosynthetic rate (P_N), especially at noon, than the older ones. At noon, all the hybrids decreased their maximal yield of photosystem 2 (PS2) photochemistry (F_v/F_m) and actual quantum yield of PS2 (Φ_{PS2}), the newer ones always showing higher values. Generally, the newer hybrids displayed higher photochemical quenching of Chl (q_P) and lower non-photochemical quenching (NPQ). The inter-hybrid differences in P_N may be owing to their differential photochemical efficiency. A midday depression in P_N occurred in all hybrids, which might be caused by serious photoinhibition or by decreased stomatal conductance. However, midday depression in P_N was more obvious in the older hybrids, especially when leaves were senescent. The higher de-epoxidation state of the xanthophylls was noted in older hybrids, which was confirmed by their larger NPQ. The newer maize hybrids did not need a strong de-epoxidation state since they had a better photosynthetic quantum conversion rate and a lower NPQ.

Additional key words: fluorescence quenching parameters; intercellular CO_2 concentration; net photosynthetic rate; stomatal conductance; *Zea mays*.

Introduction

During the past 3 to 5 decades, large increases in the average grain yield of maize have been repeatedly reported from many countries (Tollenaar 1989, Qiao *et al.* 1996). Such increases have been realized largely through genetic improvement (Khush 1999). As future yield growth will likely come from similar sources, the improvement of physiological features may process further potentials in grain yield improvement (Serageldin 1999). Although photosynthesis is the basis of the formation of crop yield, there have been contradictory conclusions over the function of the most important physiological trait, the photosynthetic rate. For example, many authors believe that no change in the net photosynthetic rate (P_N) accompanies the grain yield increases, but negative relation between P_N and grain yield was also reported (Evans 1993, Reynolds *et al.* 2000, Richards 2000). As for maize, even though some researchers reported a positive relation of grain yield and gas exchange (Dwyer and Tollenaar 1989, Dwyer *et al.* 1992, Nissanka *et al.* 1997), many others insisted on the negative relationships (Gaskel and Pearce 1981, Moll *et al.* 1994). Therefore, crop breeders are always confused by the internal reasons causing inter-hybrid differences in P_N. From this point of view, the study of gas exchange and related features in maize released in different years is particularly significant, not only because it can reveal the regulatory mechanism of photosynthesis, but also utilize the potentials of plant photosynthesis (Jiang and Xu 2000).

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Abbreviations: A: antheraxanthin; C_i: intercellular CO_2 concentration; F_v/F_m: the maximal yield of PS2 photochemistry; g_s: stomatal conductance; NPQ: non-photochemical quenching; P_N: net photosynthetic rate; PPFD: photosynthetic photon flux density; PS2: photosystem 2; q_P: photochemical quenching of chlorophyll; T: air temperature; V: violaxanthin; Z: zeaxanthin; Φ_{PS2}: actual quantum yield of PS2.

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Diurnal trend in gas exchange is often recognized as one of the best indicators in reflecting the ability of plants to maintain their photosynthetic apparatus to readily respond to environmental conditions (Geiger and Servaites 1994). Chlorophyll (Chl) fluorescence quenching analysis has been proven as a non-invasive, powerful, and reliable method to assess the changes in function of photosystem 2 (PS2) under different environments (Schreiber *et al.* 1994). Under field conditions, however, crops are always exposed to various irradiances. When plants absorb more photons than can be utilized by photosynthesis, photoinhibition always occurs as indicated by a sustained decrease in the efficiency of photon energy conversion. Xanthophyll-cycle-related thermal dissipation is the přimary approach to prevent the photosynthetic apparatus from damaging by strong irradiance under natural conditions (Demmig-Adams and Adams 1992). Therefore, the diurnal variation in gas exchange, Chl fluorescence, and amounts of xanthophyll cycle components in maize hybrids released in different years can reflect the different responses to environmental changes. In this study, we investigated the traits mentioned above in three maize hybrids released in different years and grown in the same field. The objectives of this study were to find: (*1*) the different patterns in diurnal gas exchange and Chl fluorescence parameters in different maize hybrids; (*2*) the physiological mechanisms leading to their different performance, and (*3*) the role the xanthophyll cycle may play in such process.

Materials and methods

Plants: The study was conducted in three maize (*Zea mays* L.) hybrids representing those widely cultivated in Northern China over the past five decades. They were Baimaya, Zhongdan2, and Nongda108, released in the 1950s, 1970s, and 1990s, respectively. All three hybrids were planted in a silt loam soil at the Research Farm of Shandong Agriculture University, Shandong province, on 28 June 2003. Plants were arranged in two randomized complete blocks, 800 cm long and 270 cm wide. The plant population density was 4.5 plants per m^2. All the maize hybrids received the same agricultural management. The plots were fertilized with 16.2 g m^{-2} urea at sowing and 26.3 g m^{-2} urea on 27 July. Irrigation was not applied because of the plentiful rain that year. The plots were periodically sprayed with fungicides to avoid disease damage. Weeds were hand-pulled periodically.

Gas exchange: Only flag leaves were determined in the maize growth season of 2003 (June to September). Actual measurements were done at the 4th and 5th week after flowering. Gas exchanges, photosynthetic photon flux density (PPFD), and leaf temperature were simultaneously recorded every two hours from 06:00 to 18:00 on clear days with a portable photosynthetic system (*CIRAS-1, PP Systems*, UK). The leaves for experiments were all fully exposed and oriented to normal irradiation during measurements to find gas exchange at the highest possible PPFD. Five replications were done for each treatment at each time.

Chl fluorescence was measured using a portable fluorometer (*FMS 2, Hansatech*, UK). Initial fluorescence (F_0) was recorded on leaves adapted to dark for 5 min. A single saturating radiation pulse was applied to obtain the maximum Chl fluorescence (F_m). Steady state Chl fluorescence (F_s) was determined under actinic irradiation. Saturating pulses were utilized in order to obtain F_m' following at each actinic irradiation. When the actinic radiation was removed, then the minimal Chl fluorescence in the light-adapted state (F_0') was determined by irradiating the leaf disk for 3 s with far-red radiation. The maximum efficiency of PS2 photochemistry (F_v/F_m), actual quantum yield of PS2 (Φ_{PS2}), and non-photochemical quenching (NPQ) were calculated according to Demmig-Adams *et al.* (1996).

Pigment analysis: The carotenoids of xanthophyll cycle were determined according to Thayer and Björkman (1990) with some modifications. The samples were collected when gas exchange and Chl fluorescence were measured. Pigments were extracted with 100 % acetone before separating in an HPLC system (*Waters*, USA) at room temperature. The mobile phase was composed of three solvents: acetonitrile, methanol, and methylene chloride. To achieve a better separation of the xanthophyll cycle pigments, the elution program was formulated as follows: At first, during 0–4 min a mixture of solvents, acetonitrile – methanol – Tris-HCl (72 : 8 : 3, v:v:v) was run, followed by 2.5 min of linear gradient; Then the mixed solvent was changed to methanol and hexane (5 : 1) for 10 min between the two sample analyses. Finally, the column was re-equilibrated for 10 min with the mixed solvent used in the first period (0–4 min). The de-epoxidation of xanthophyll cycle was calculated in percent using a peak area, as follows:

$$(A+Z) = (A_{\text{peak area}} + Z_{\text{peak area}})/(V_{\text{peak area}} + A_{\text{peak area}} + Z_{\text{peak area}})$$

Data analysis: Analysis of variance (ANOVA) of leaf traits was carried out on each measurement and the results were analyzed by SPSS (10.0 for *Windows*). The least significant differences (LSD) between the means were estimated at 95 % confidence level. Unless indicated otherwise, significant differences among different plants are given at $p<0.05$. Calculations and linear regressions were performed using a *SigmaPlot 8.0* program.

Results

Gas exchange: In general, P_N of Nongda108 was the highest and Baimaya the lowest among the three hybrids. P_N of all hybrids did not reach the maximum value at 12:00 when PPFD peaked (Figs. 1*A,B* and 2*A,B*). The P_N values of all hybrids found at 12:00 were lower than those at 14:00. Moreover, Baimaya at the 4th week after flowering and Baimaya and Zhongdan2 at the 5th week after flowering had even lower P_N values at 12:00 than at 10:00. Changes in stomatal conductance (g_s) of the three hybrids at both stages were similar (Fig. 2*C,D*), *i.e.* increased from 06:00 to 14:00 and then decreased gradually. The changes in intercellular CO_2 concentration (C_i) were just contrary to those of P_N (Fig. 2*E,F*). However, there were no significant differences in g_s and C_i among all hybrids. A close positive correlation was observed between P_N and g_s for measurements taken at the 4th week after flowering and 5th week after flowering (Fig. 3*A*).

Chl fluorescence: F_v/F_m of Nongda108 was higher than that of other hybrids, with Zhongdan2 being a little higher than Baimaya at both stages (Fig. 4*A,B*). Midday reduction in Zhongdan2 at the 5th week after flowering was more serious than that of the 4th week after flowering. At both stages, the diurnal trends of Φ_{PS2} and photochemical quenching of chlorophyll (q_P) were similar to that of F_v/F_m (Fig. 4*C–F*), except that the reductions at noon were more obvious. However, the non-photochemical quenching (NPQ) at both stages was just contrary to that of Φ_{PS2} and q_P (Fig. 4*G,H*). At noon, NPQ of all three hybrids increased obviously at both stages with Baimaya being the highest. The midday increase in NPQ of Zhongdan2 was more obvious at the 5th week than at the 4th week after flowering.

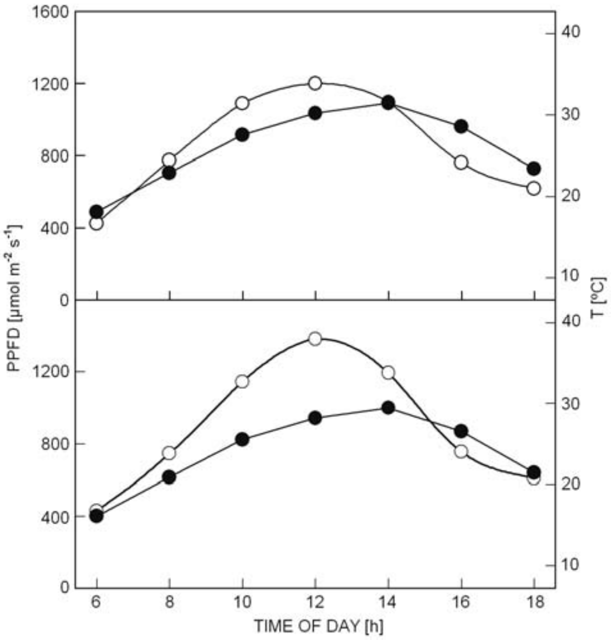

Fig. 1. Diurnal trends of photosynthetic photon flux density (PPFD, ○) and air temperature (T, ●) of maize hybrids released in different years at the 4th week after flowering (*A*) and the 5th week after flowering (*B*).

Xanthophyll cycle components: The diurnal trends of (A+Z)/(V+A+Z) were just similar to that of NPQ (Fig. 5), which increased from morning to noon and then decreased to the morning level in the afternoon. For example, the maximum of (A+Z)/(V+A+Z) at the 4th week after flowering was 0.43 in Baimaya, while it was 0.36 and 0.32, respectively, in Zhongdan2 and Nongda108. At the 5th week after flowering, the maximum values were 0.45, 0.42, and 0.35 for Baimaya, Zhongdan2, and Nongda108, respectively.

Discussion

Leaf P_N in new crops can keep high throughout the days in the growth season, especially after thesis. Such high P_N makes the base for the formation of yields. In our study, as a whole, the maize hybrid released in 1990s had higher P_N than those released in 1970s and 1950s during the days of growth season, especially at noon (Fig. 2*A,B*).

Chl fluorescence parameters can reflect the PS2 response to the environmental conditions (Schreiber *et al.* 1994). Genetic variation in photochemical activity has already been observed in maize (Krebs *et al.* 1996, Holá *et al.* 1999). In our study the newer hybrid always had higher maximal yield of PS2 photochemistry (F_v/F_m) (Fig. 4*A,B*) and actual quantum yield of PS2 (Φ_{PS2}) (Fig. 4*C,D*) than the older ones. So we might believe that the photochemical efficiency of the newer hybrids was higher than that of the older ones especially at noon. q_P reflects the proportion of photon energy absorbed by the PS2 light-harvesting complex (Manoj and Prasanna 1995). We noted that the diurnal trends of q_P (Fig. 4*E,F*) were similar to that of Φ_{PS2}. It decreased from morning to noon and recovered during the afternoon. Nevertheless, q_P of Nongda108 was always higher than the other ones. The decrease in q_P may indicate a decrease in the proportion of the closed PS2 reaction centres (RCs) or in the proportion of the reduced state of Q_A (Genty *et al.* 1989). The diurnal trends of NPQ and the thermal dissipation of the energy absorbed by the antenna pigment (Alexander and Peter 1995) were just contrary to that of q_P on both stages (Fig. 4*G,H*). Higher q_P and lower NPQ of the newer hybrids indicated that the fraction of the open PS2 RCs has been improved, so more excitation energy captured by the antenna pigment can be used to propel the photosynthetic electron transfer (Zhang *et al.* 1996), with more NADPH and ATP being supplied for the CO_2 assimilation (Harbinson *et al.* 1990). Therefore, the higher P_N values of the newer hybrids were probably, at least in part, due to their higher photochemical efficiency.

Midday depression of leaf P_N is usually found in C_3 plants such as rice (Ishihara and Saitoh 1987), soybean (Huck *et al.* 1983), *etc*. We also found midday depression of P_N in the C_4 maize hybrids (Fig. 2*A*,*B*), although it was not so typical. Many researchers reported that the main cause for the reduction in photosynthetic rate was largely due to stomatal closure (Hirasawa *et al.* 1989, Wakabayashi *et al.* 1996). The close positive correlation between P_N and g_s (Fig. 3*A*) indicates that the midday depression of P_N in maize might be due to stomatal closure. But the increase in g_s was always accompanied by a decrease in C_i (Fig. 2*B*,*C*). The low C_i values at high g_s values suggested that the midday depression of P_N was not caused primarily by the lower g_s, but rather by non-stomatal factors. Leverenz *et al.* (1990) reported that a decrease in maximum carboxylation rate occurs at a deep photoinhibition while the decrease in initial quantum yield starts at the primary stage of photoinhibition. In this study, F_v/F_m and Φ_{PS2} of three maize hybrids released in different years all decreased with the increased PPFD in the morning, then recovered during the afternoon when PPFD decreased (Fig. 4). These reversible changes suggested that a down-regulation of PS2 might happen in

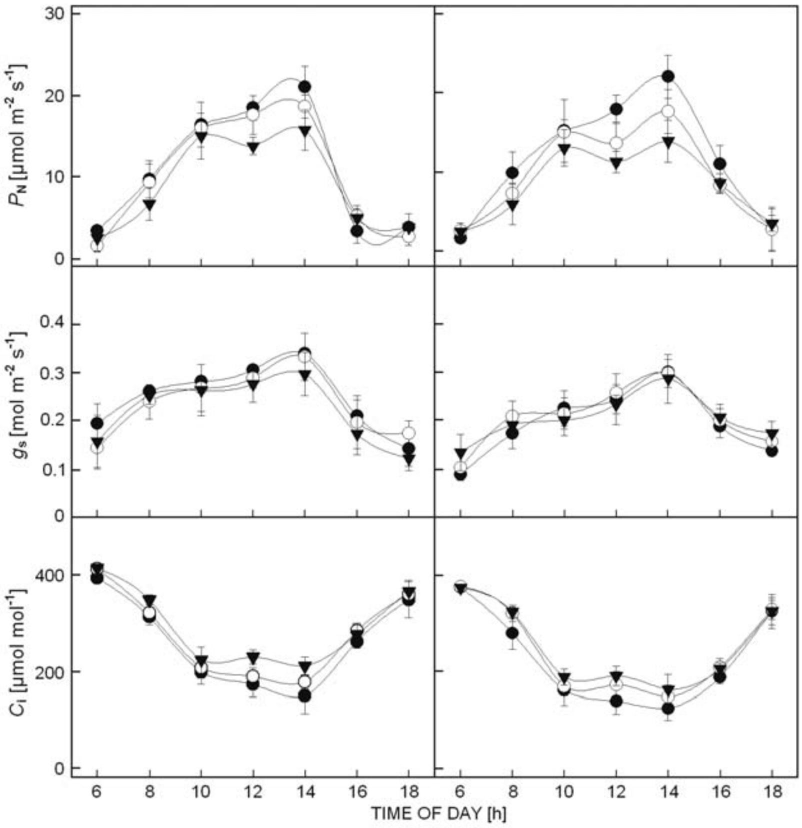

Fig. 2. Diurnal trends of net photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) of maize hybrids released in different years at the 4th week after flowering (*A*,*C*,*E*) and the 5th week after flowering (*B*,*D*,*F*). ●: 1990s hybrids; ▲: 1970s hybrids; ■: 1950s hybrids. SEs are shown.

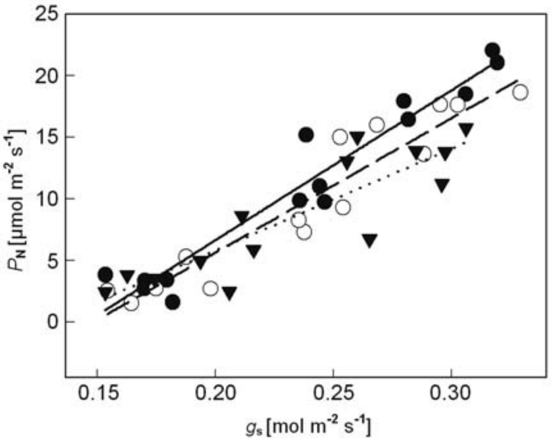

Fig. 3. Relationship between net photosynthetic rate (P_N) and stomatal conductance (g_s) of maize hybrids released in different years. ●: 1990s hybrids; ▲: 1970s hybrids; ■: 1950s hybrids.

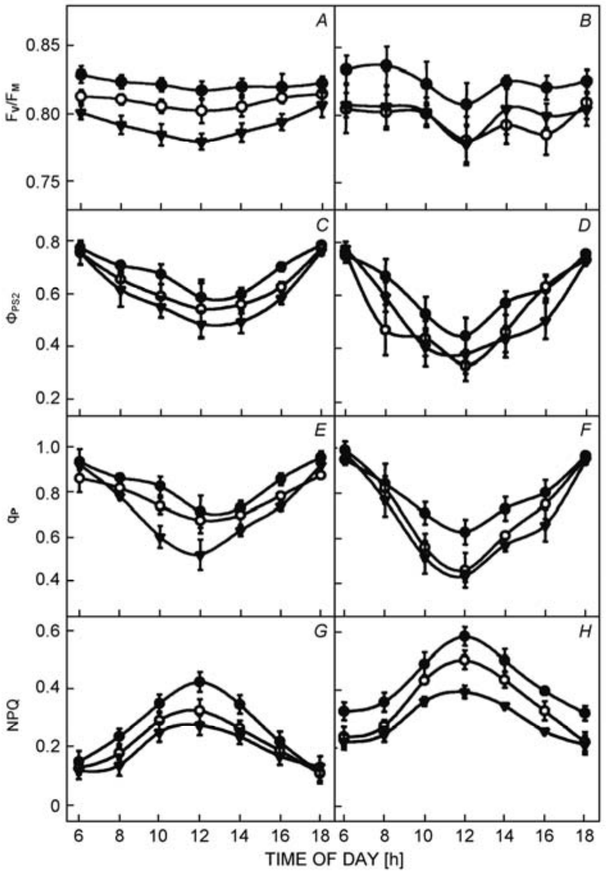

Fig. 4. Diurnal trends of PS2 primarily chemical efficiency (F_v/F_m), actual quantum yield of PS2 (Φ_{PS2}), photochemical quenching of chlorophyll (q_P), and non-photochemical quenching of chlorophyll (NPQ) of maize hybrids released in different years at the 4th week after flowering (*A,C,E,G*) and at the 5th week after flowering (*B,D,F,H*). ●: 1990s hybrids; ▲: 1970s hybrids; ■: 1950s hybrids. SEs are shown.

senescent leaves. Such a response may reflect a protective or regulatory mechanism in avoiding photodamage to the photosynthetic apparatus under excess irradiance (Demmig-Adams and Adams 1992). So, the midday depression of P_N of the three maize hybrids (Fig. 2*A,B*) may be caused by a serious photoinhibition. We also found that the midday depressions were more obvious (Fig. 2*A,B*) when the Chl content declined sharply, *i.e.* Baimaya at the 4th week after flowering and Zhongdan2 at the 5th week after flowering (Ding *et al.* 2005). Since the loss of Chl is a typical phenomenon for leaf senescence, we may say that the midday depression in P_N can be more obvious at serious senescent leaves, possibly because of a more serious photoinhibition. The different reduction of P_N at midday of the different hybrids might be owing to their different rate of senescence.

During the evolution process, plants have developed many endogenous adaptive reaction systems to protect their photosynthetic apparatus against photoinhibition (Krause 1988). Non-radiative energy dissipation mediated *via* the xanthophyll cycle occurs in the light-harvesting pigment-protein complexes of PS2 (Gilmore 1997). Through this mechanism, excitation energy can be dissipated harmlessly into heat before reaching the RC core. Our results suggested that the xanthophyll cycle played an important role in dissipating excess photon energy when the maize hybrids were exposed to high irradiance. Such role was especially evident in the senescent leaves (Fig. 5). The higher de-epoxidation state was found in the older hybrids, which was confirmed by their bigger values of NPQ. Hence the newer maize hybrids need not have a strong de-epoxidation state of the xanthophyll cycle since they have a better photosynthetic quantum conversion rate and also a lower state of NPQ.

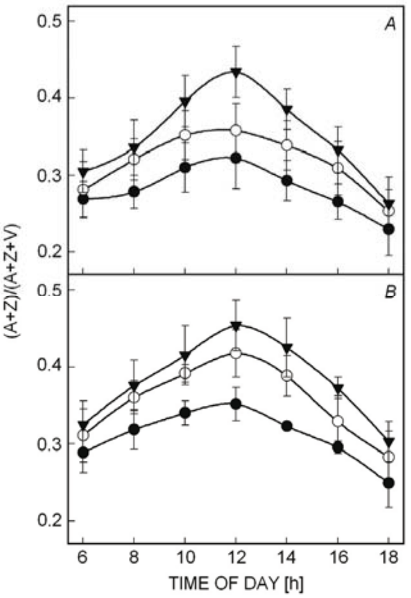

Fig. 5. Diurnal trends of the de-epoxidation extent of the xanthophyll cycle of maize hybrids released in different years at the 4th week after flowering (*A*) and the 5th week after flowering (*B*). ●: 1990s hybrids; ▲: 1970s hybrids; ■: 1950s hybrids. SEs are shown.

In conclusion, the newer maize hybrids always had higher P_N than the older ones during the growth day, especially at noon. Such phenomenon might be explained by the different photochemical efficiency of the light reaction system. Midday depressions in P_N were found in all hybrids released at different years, which were caused by either serious photoinhibition or low g_s. Moreover, midday depression in P_N was more obvious in the older hybrids especially when leaves were senescent. The higher de-epoxidation state in the older hybrids may help to avoid photoinhibitory damage when exposed to excessive photon energy. The newer maize hybrids do not need a strong de-epoxidation state of the xanthophyll cycle since they have a better photosynthetic quantum conversion rate and a lower NPQ.

References

Alexander, V.R., Peter, H.: Regulation of non-photochemical quenching of chlorophyll fluorescence in plants. – Aust. J. Plant Physiol. **22**: 221-230, 1995.

Demmig-Adams, B., Adams, W.W., III: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 599-626, 1992.

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

Ding, L., Wang, K.J., Jiang, G.M., Liu, M.Z., Niu, S.L., Gao, L.M: Post-anthesis changes in photosynthetic traits of maize hybrids released in different years. – Field Crops Res. **93**: 108-115, 2005.

Dwyer, L.M., Stewart, D.W., Tollenaar, M.: Analysis of maize leaf photosynthesis under drought stress. – Can. J. Plant Sci. **72:** 477-481, 1992.

Dwyer, L.M., Tollenaar, M.: Genetic improvement in photosynthetic response of hybrid maize cultivars, 1959 to 1988. – Can. J. Plant Sci. **69**: 81-91, 1989.

Evans, L.T.: Crop Evolution, Adaptation and Yield. – Cambridge University Press, New York 1993.

Gaskel, M.L., Pearce, R.B.: Growth analysis of maize hybrids differing in photosynthetic capability. – Agron. J. **73**: 817-821, 1981.

Geiger, D.R., Servaites, J.C.: Diurnal regulation of photosynthetic carbon metabolism in C_3 plants. – Annu. Rev. Plant Physiol. Plant mol. Biol. **45**: 235-256, 1994.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. biophys. Acta **990**: 87-92, 1989.

Gilmore, A.M.: Mechanistic aspects of xanthophyll cycle-dependent photo-protection in higher plant chloroplasts and leaves. – Physiol. Plant. **99**: 197-209, 1997.

Harbinson, J., Genty, B., Baker, N.R.: The relationship between CO_2 assimilation and electron transport in leaves. – Photosynth. Res. **25**: 313-324, 1990.

Hirasawa, T., Iida, Y., Ishihara, K.: [Dominant factors in reduction of photosynthetic rate affected by air humidity and leaf water potential in rice plants.] – Jap. J. Crop Sci. **58**: 383-389, 1989. [In Jap.]

Holá, D., Kočová, M., Körnerová, M., Sofrová, D., Sopko, B.: Genetically based differences in photochemical activities of isolated maize (*Zea mays* L.) mesophyll chloroplasts. – Photosynthetica **36**: 187-197, 1999.

Huck, M.G., Ishihara, K., Peterson, C.M., Ushijima, T.: Soybean adaptation to water stress at selected stages of growth. – Plant Physiol. **73**: 422-427, 1983.

Ishihara, K., Saitoh, K.: [Diurnal courses of photosynthesis, transpiration, and diffusive conductance in the single-leaf of the rice plants grown in the paddy field under submerged condition.] – Jap. J. Crop Sci. **56**: 8-17, 1987. [In Jap.]

Jiang, H., Xu, D.-Q.: Physiological basis of the difference in net photosynthetic rate of leaves between two maize strains – Photosynthetica **38**: 199-204, 2000.

Khush, G.S.: Green revolution: preparing for the 21st century. – Genome **42**: 646-655, 1999.

Krause, G.H.: Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. – Physiol. Plant. **74**: 566-574, 1988.

Krebs, D., Synková, H., Avratovščuková, N., Kočová, M., Šesták, Z.: Chorophyll fluorescence measurements for genetic analysis of maize cultivars. – Photosynthetica **32**: 505-608, 1996.

Leverenz, J.W., Falk, S., Pilström, C.M., Samuelsson, G.: The effects of photoinhibition on the photosynthetic light-response curve of green plant cells (*Chlamydomonas reinhardtii*). – Planta **182**: 161-168, 1990.

Manoj, K.J., Prasanna, M.: Probing photosynthetic performance by chlorophyll *a* fluorescence analysis and interpretation of fluorescence parameters. – J. Sci. Ind. **54**: 155-174, 1995.

Moll, R.H., Jackson, W.A., Mikkelsen, R.L.: Recurrent selection for maize grain yield: dry matter and nitrogen accumulation and partitioning changes. – Crop Sci. **34**: 874-881, 1994.

Nissanka, S.P., Dixon, M.A., Tollenaar, M.: Canopy gas exchange response to moisture stress in old and new maize hybrids. – Crop Sci. **37**: 172-181, 1997.

Qiao, C.G., Wang, Y.J., Guo, H.A., Chen, X.J., Liu, J.Y., Li, S.Q.: A review of advances in maize production in Jilin Province during 1974-1993. – Field Crops Res. **47**: 65-75, 1996.

Reynolds, M.P., Van Ginkel, M., Ribaut, J.-M.: Avenues for genetic modification of radiation use efficiency in wheat. – J. exp. Bot. **51**: 459-473, 2000.

Richards, R.A.: Selectable traits to increase crop photosynthesis and yield of grain crops. – J. exp. Bot. **51**: 447-458, 2000.

Schreiber, U., Bilger, W., Neubauer, C.: Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis. – In: Schulze, E.-D., Caldwell, M.M. (ed.): Ecophysiology of Photosynthesis. Pp. 49-70. Springer Verlag, Berlin 1994.

Serageldin, I.: Biotechnology and food security in the 21st century. – Science **285**: 387-389, 1999.

Thayer, S.S., Björkman, O.: Leaf xanthophyll content and composition in sun and shade determined by HPLC. – Photosynth. Res. **23**: 331-343, 1990.

Tollenaar, M.: Genetic improvement in grain yield of commercial maize hybrids grown in Ontario from 1959 to 1988. – Crop Sci. **29**: 1365-1371, 1989.

Wakabayashi, K., Hirasawa, T., Ishihara, K.: [Analysis of photosynthesis depression under low leaf water potential by comparison of CO_2 exchange and O_2 evolution rates.] – Jap. J. Crop Sci. **65**: 590-598, 1996. [In Jap.]

Zhang, Q.D., Lu, C.M., Feng, L.J., Lin, S.Q., Kuang, T.Y., Bai, K.Z.: Effect of elevated CO_2 on the primary conversion of light energy of alfafa photosynthesis. – Acta bot. sin. **38**: 77-82, 1996.