BRIEF COMMUNICATION

Midday photoinhibition of two newly developed super-rice hybrids

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Abstract

Super-rice hybrids are two-line hybrid rice cultivars with 15 to 20 % higher yields than the traditional three-line hybrid rice cultivars. Response of photosynthetic functions to midday photoinhibition was compared between seedlings of the traditional hybrid rice (*Oryza sativa* L.) Shanyou63 and two super-rice hybrids, Hua-an3 and Liangyoupeijiu. Under strong midday sunlight, in comparison with Shanyou63, the two super-rice hybrids were less photoinhibited, as indicated by the lower loss of the net photosynthetic rate (P_N), the quantum yield of photosystem 2 (Φ_{PS2}), and the maximum and effective quantum yield of PS2 photochemistry (F_v/F_m and F_v'/F_m'). They also had a much higher transpiration rate. Hence the super-rice hybrids could protect themselves against midday photoinhibition at the cost of water. The photoprotective de-epoxidized xanthophyll cycle components, antheraxanthin (A) and zeaxanthin (Z), were accumulated more in Hua-an3 and Liangyoupeijiu than in Shanyou63, but the size of xanthophyll cycle pool of the seedlings was not affected by midday photoinhibition. Compared to Shanyou63, the super-rice hybrids were better photoprotected under natural high irradiance stress and the accumulation of Z and A, not the size of the xanthophyll pool protected the rice hybrids against photoinhibition.

Additional key words: chlorophyll fluorescence; cultivar differences; net photosynthetic rate; *Oryza*; photosystem 2; quantum yield; transpiration rate; xanthophyll cycle.

From the 1960s till now world grain harvest yields have continued to rise, however, the increase in rice yield levelled off in the 1990s. While the population continues to grow, *per capita* production has flattened (Mann 1999). To solve the problem of the plateau of growth of grain yields and feed the growing population, China has further tapped the imperfectly understood heterosis (first generation of hybrid plants is typically more vigorous and productive than either parent) and developed the super-rice, whose yield is elevated on average by 15 to 20 %, comparing with the traditional hybrid rice. The theory of the breed system is based on morphological characters of long, narrow, and very erect top leaves. Prof. Longping Yuan (director of China's National Hybrid Rice Research and Development Center in Changsha, Hunan Province) believes that this configuration captures sunlight more effectively. Most of the current research carried out on hybrid rice was mainly done by the Chinese and focused on the properties of heterosis and morphology (Khan *et al.* 1997, 1998). During the past several years, Ji and Jiao published a series of papers on photosynthesis and photoinhibition of the hybrid rice varieties and argued that *japonica* rice cultivars are better protected from photoinhibition than the *indica* ones (Ji *et al.* 1994, 2000, Jiao *et al.* 1994, Ji and Jiao 1999, 2001, Jiao and Ji 2001). We studied the photosynthetic characteristics of super-rice cultivars (Wang *et al.* 2000). But no work related to photoinhibition and photoprotection of these super-rice hybrids has been done yet. In this article we describe their midday photoinhibition under field conditions.

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Abbreviations: A, antheraxanthin; DES, de-epoxidation state; *E*, transpiration rate; F_0 and F_0', minimal fluorescence with all PS2 reaction centres open in the dark- and light-adapted state, respectively; F_m and F_m', maximal fluorescence with all PS2 reaction centres closed in the dark- and light-adapted state, respectively; F_v/F_m, maximum quantum yield of PS2 photochemistry; F_v'/F_m', effective quantum yield of PS2 photochemistry; P_N, net photosynthetic rate; PS, photosystem; WUE, water use efficiency; Z, zeaxanthin; Φ_{PS2} quantum yield of PS2 electron transport.

Photosynthesis cannot proceed in the absence of photons, but no leaf can utilize all of those absorbed by antenna systems during exposure to full sunlight. Excessive photons are dangerous to plants because they can inhibit photosynthesis and may lead to photooxidative destruction of the photosynthetic apparatus (photoinhibition) (Baker and Bowyer 1994, Foyer and Noctor 2000). Photoinhibition can occur in the field under natural conditions (Powles 1984, Demmig-Adams and Adams 1992, 1996b, Long *et al.* 1994, Anderson *et al.* 1997, Murchie *et al.* 1999). Leaves have developed various mechanisms to deal with excess photon energy (Demmig-Adams 1990, Barber and Andersson 1992, Demmig-Adams and Adams 1992, Chow 1994, Horton *et al*. 1994, Anderson *et al.* 1997, Niyogi 1999).

As photosynthesis is the basis of crop yields, photoinhibition has an adverse effect on photosynthesis and the accumulation of dry biomass, which can lead to a carbon assimilation decrease of about 10 % (Long *et al.* 1994). The ability to disperse excessive photon energy, *i.e*. resistance to photoinhibition of crops, will significantly affect the grain yield.

The main objective of our study was to investigate the responses of photosynthetic functions of the super-rice to photoinhibition under field conditions. For this purpose, we studied net photosynthetic rate (P_N), chlorophyll (Chl) fluorescence, and the xanthophyll cycle of two super-rice hybrids, Hua-an3 and Liangyoupeijiu (both have a yield of more than 10 500 kg per ha), and the traditional hybrid rice, Shanyou63 (with a yield of about 7 500~8 250 kg per ha).

Seeds of Hua-an3 (X07S×Zihui100, *indica*), Liangyoupeijiu (Peiai64S×93-11, *indica-japonica* hybrid), and Shanyou63 (Zhenshan97A×Minghui63, *indica*) were presoaked in a water bath at 30 °C over night and then covered with wet gauze cloth for germination at 25 ^oC in an irradiated incubator (Guangdong China) with an irradiance of 40 µmol(photon) m^{-2} s^{-1}. The germinated seeds were planted in 30-cm high pots with a diameter of 25 cm and grown outdoors under natural conditions. The seedlings were about 20 cm in height after 30 d. The second leaf counted down from the top was used for experiments and all the parameters were measured with at least five repetitions.

Photoinhibition experiments on the three rice hybrids were carried out at 14:00. Plants received around 1 800 µmol(photon) m^{-2} s^{-1} (35 ^oC); field photoinhibition was at its most serious point during the day. In contrast, all controlled parameters were measured predawn, unless otherwise specified. P_N and transpiration rate, E were measured using an open-system portable CO_2/H_2O analyzer (*Ciras-1*, *PP-Systems*, UK). Water use efficiency (WUE) was estimated as P_N/E. Chl fluorescence was measured on intact leaves by using a portable fluorometer *PAM-2000* (*Walz*, Effeltrich, Germany) with a notebook computer and the data collection software *DA-2000* according to Genty *et al.* (1989). First, a very low [<0.1 µmol(photon) m^{-2} s^{-1}] modulated measuring radiation was applied to the dark-adapted (20 min) leaf to determine the minimal fluorescence level (F_0). This was followed by a 0.8-s saturating pulse at 8 000 µmol(photon) m^{-2} s^{-1} to determine the maximal fluorescence level (F_m). Then, the leaf was continuously irradiated with "actinic light" of 200 µmol(photon) m^{-2} s^{-1} using a halogen lamp until the steady-state fluorescence (F_s) was reached. A second saturating pulse with the same properties as the former one was applied to the leaf to determine the maximal fluorescence level in the light-adapted state (F_m'). The "actinic light" was then turned off and the minimal fluorescence level in the light-adapted state (F_0') was determined by irradiating the leaf for 3 s with far-red radiation. The maximal and effective quantum yield of photosystem 2 (PS2) photochemistry (F_v/F_m F_v'/F_m') and the quantum yield of PS2 electron transport (Φ_{PS2}) were calculated as $F_v/F_m = (F_m - F_0)/F_m$, $F_v'/F_m' = (F_m' - F_0')/F_m'$, and $\Phi_{PS2} = (F_m' - F_s)/F_m'$, respectively, according to Genty *et al.* (1989).

For pigment analysis, leaf segments were quickly frozen in liquid nitrogen and then stored at –80 ^oC until analyzed. Pigments were extracted in 100 % acetone, and the extracts were filtered through a 0.45 µm membrane filter before HPLC injection. Photosynthetic pigments were separated and quantified by HPLC (*Waters600*, Japan) following the protocol of Xu *et al.* (1999). De-epoxidation state (DES) was calculated as (zeaxanthin, Z + 1/2 antheraxanthin, A)/(Z + A + violaxanthin, V).

Under midday strong sunlight, E of the two super-rices increased by 5 and 12 %, respectively (Fig. 1*A*), while that of Shanyou63 decreased by 23 %. P_N of the two super-rices decreased by 15 and 19 %, respectively, compared with their highest value of the day (at 10:00), and that of Shanyou63 decreased by 26 % (Fig. 1*B*). WUE (Fig. 1*C*) of Liangyoupeijiu and Hua-an3 dropped significantly (by 19 and 28 %, respectively), while in Shanyou63 it slightly decreased (by 4 %). The F_v/F_m (Fig. 1*D*), F_v'/F_m' (Fig. 1*E*), and Φ_{PS2} (Fig. 1*F*) values of all three rice hybrids decreased significantly, but a greater decrease was observed in Shanyou63.

When midday photoinhibition occurred, the content of V (Fig. 2*A*) decreased significantly and was de-epoxidased to A (Fig. 2*B*) and Z (Fig. 2*C*) through the so-called xanthophyll cycle, while the total xanthophyll cycle pool (V+A+Z, Fig. 2*D*) was not affected. The increase in A and Z contents of the two super-rices was greater than in Shanyou63, and the overall content of the photoprotective xanthophylls (A+Z, Fig. 2*F*) was much higher than in Shanyou63. Further, the increasing degree of de-epoxidation state (DES) in Liangyoupeijiu and Hua-an3 was higher than in Shanyou63 (Fig. 2*E*). This agrees with the results obtained for other high-yielding rice mutants (Dai *et al*. 2003, Lin *et al*. 2003a,b).

Elevated E is one protective way in which plants respond to strong midday irradiation. In this way, plants can dissipate part of the excess energy absorbed by leaves and lower the leaf surface temperature, thus protecting from strong irradiance and high environmental temperature. When subjected to strong sunlight at midday, *E* of Liangyoupeijiu and Hua-an3 increased by 5 and 12 %, respectively, while that of Shanyou63 decreased by 23 %. At the same time, P_N of super-rice decreased a little, while that of Shanyou63 decreased significantly. Elevated *E* was taken as one protective response of plants when subjected to strong sunlight and high temperature, but what led to the decrease of *E* of Shanyou63 under midday strong irradiance? We suggest that Shanyou63 is seriously photoinhibited under strong midday sunlight and high temperature, the stomata partly close, and this leads to the decrease in *E*. The super-rice hybrids, however, are well protected at the cost of water. Their increased *E* under the strong midday sunlight indicates relatively open stomata which may keep a relatively high P_N.

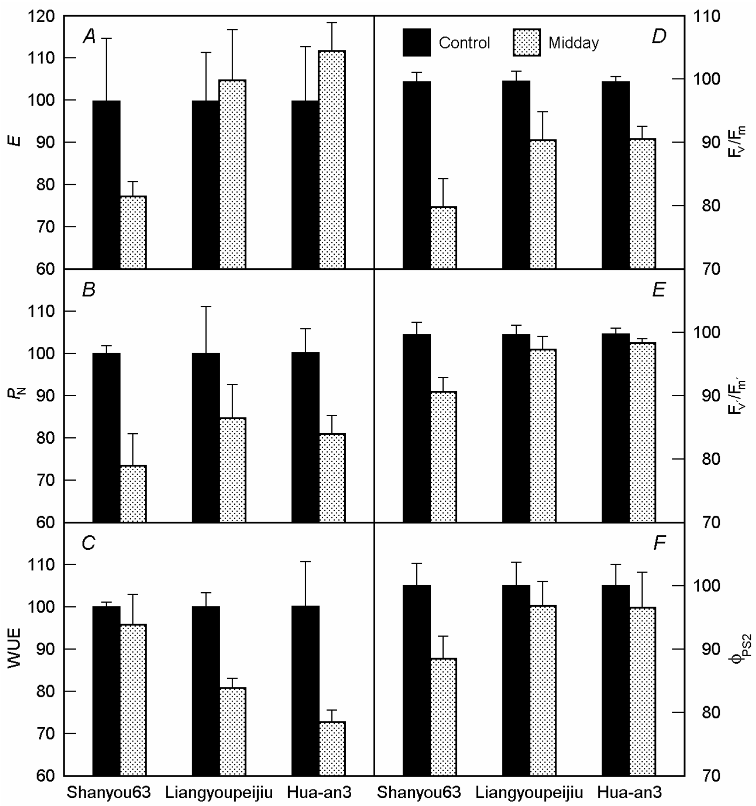

Fig. 1. Response of gas exchange and chlorophyll fluorescence characteristics to midday photoinhibition: (*A*) transpiration rate, *E*; (*B*) net photosynthetic rate, P_N; (*C*) water use efficiency, WUE; (*D*) F_v/F_m; (*E*) F_v'/F_m', and (*F*) Φ_{PS2}. Controls were measured at 10:00 when the photosynthetic apparatus was most active during the day and were set as 100 for contrast. Means ± S.D. of five replicates. The 100 values of *E*, P_N, WUE, F_v/F_m, F_v'/F_m', and Φ_{PS2} for Shanyou63 were 11.14±1.65 mmol(H_2O) m^{-2} s^{-1}, 13.39±0.23 µmol(CO_2) m^{-2} s^{-1}, 1.20±0.02 mmol(CO_2) mol^{-1}(H_2O), 0.816±0.012, 0.595±0.012, and 0.359±0.012, respectively. Values for Liangyoupeijiu and Hua-an3 were in the error range of ±2–5 % (gas exchange) or ±3 % (fluorescence) different from those of Shanyou63.

F_v/F_m represents the maximum quantum yield of PS2 photochemistry (Kitajima and Butler 1975) and is often used as a characteristic of potential yield of PS2 photochemical reactions (Krause and Weis 1991). Without stress, the maximum value of F_v/F_m is almost constant for many different plant species and ecotypes (Björkman and Demmig 1987), but is remarkably reduced when subjected to stresses, thus was often used for estimating the extent of photoinhibition and other stresses (Schreiber and Bilger 1987, van Kooten and Snel 1990). The reversible midday depression of F_v/F_m is termed dynamic photoinhibition (Dodd *et al.* 1998). The dynamic photoinhibition of Shanyou63 was more seriously affected than that of Liangyoupeijiu and Hua-an3 (Fig. 1). Furthermore, the decrease in F_v'/F_m' and Φ_{PS2} was higher in Shanyou63 than in the super-rices. From these findings together with the results of gas exchange we conclude that Shanyou63 seedlings were more photoinhibited than both super-hybrids. This was consistent with results obtained with flag leaves (Wang *et al.* 2000).

The xanthophyll cycle is important in photoprotection (Horton *et al.* 1996, Niyogi 1999, Müller *et al.* 2001). When exposed to continuous high irradiance, the excess excitation of PS2 generates a high ΔpH across the thylakoid membrane and activates the inter-conversion in the xanthophyll cycle (Horton *et al.* 1996, Niyogi 1999, 2000) resulting in a relative increase in Z content on expense of V. The xanthophyll cycle pool of the three rice hybrids remained unchanged under midday photoinhibition (Fig. 2), while the contents of de-epoxidized components Z and even A increased significantly, suggesting that not the total xanthophyll pool, but the accumulation of its Z and A are effective in photoprotection of rice under midday photoinhibition. As discussed above, the decreased *E* in Shanyou63 was a result of closed stomata and subsequently inhibited its CO_2 fixation, which should lead to a higher ΔpH and conversion of V to A and Z. Fig. 2 shows that much more A and Z accumulated in Liangyoupeijiu and Hua-an3, hence the xanthophyll cycle worked more efficiently in the two super-rice hybrids, and thus made them better photoprotected.

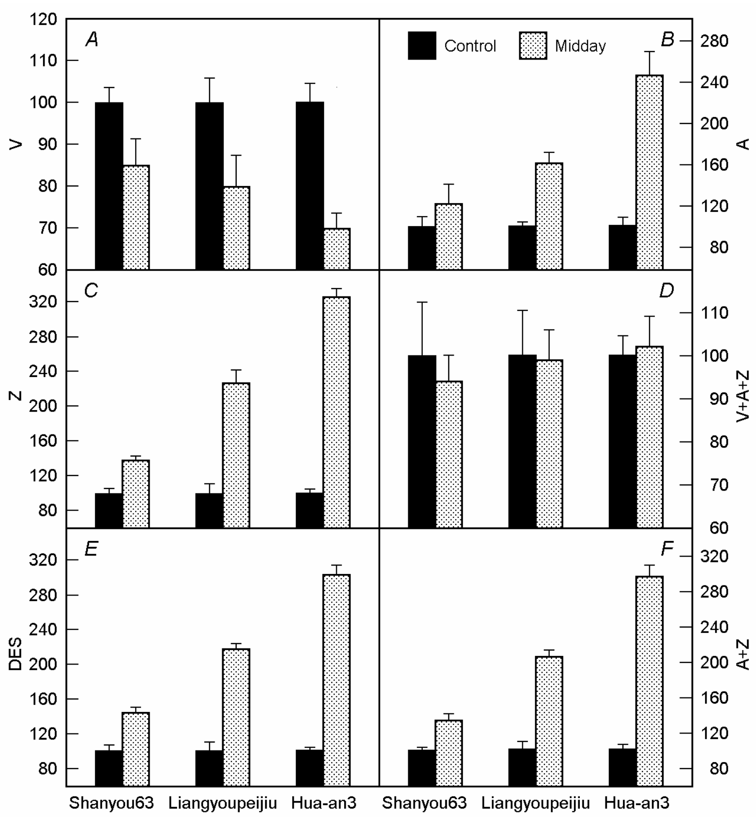

Fig. 2. Response of xanthophyll cycle to midday photoinhibition. (*A*) violaxanthin, V; (*B*) antheraxanthin, A; (*C*) zeaxanthin, Z; (*D*) xanthophyll cycle pool (V+A+Z); (*E*) de-epoxidation state (DES), and (*F*) overall content of the de-epoxidized xanthophyll cycle components (A+Z). Controls were measured predawn and were set as 100. Means ± S.D. of five replicates. 100 of V, A, and Z for Shanyou63 were 6.650±0.245, 0.433±0.039, and 1.120±0.068 μmol m^{-2}(leaf area). Those for Liangyoupeijiu and Hua-an3 were in the error range of less than ±7–11 % different from Shanyou63.

When subjected to midday sunlight stronger than the saturation irradiance, photoinhibition appeared in all three cultivars, but the two super-rice hybrids were better photoprotected by the expense of water and the more efficiently working xanthophyll cycle. This feature will surely contribute to their higher yield. For instance, in China, Liangyoupeijiu had a yield by 18.2~32.1 % higher than Shanyou63 in the 121 experimental units of 13 provinces in 1998, while Hua-an3 had a 23.4~33.8 % higher yield. Comparing with Liangyoupeijiu, Hua-an3 has a relatively higher yield which might relate to the better performance of its xanthophyll cycle.

References

Anderson, J.M., Park, Y.-I., Chow, W.S.: Photoinactivation and photoprotection of photosystem II in nature. – Physiol. Plant. **100**: 214-223, 1997.

Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis from Molecular Mechanisms to the Field. – BIOS Scientific Publishers, Oxford 1994.

Barber, J., Andersson, B.: Too much of a good thing: light can be bad for photosynthesis. – Trends biochem. Sci. **17**: 61-66, 1992.

Björkman, O., Demmig, B.: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. – Planta **170**: 489-504, 1987.

Chow, W.S.: Photoprotection and photoinhibitory damage. – Adv. mol. Cell Biol. **10**: 151-196, 1994.

Dai, X., Xu, X., Lu, W., Kuang, T.: Photoinhibition characteristics of a low chlorophyll *b* mutant of high yield rice. – Photosynthetica **41**: 57-60, 2003.

Demmig-Adams, B.: Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. – Biochim. biophys. Acta **1020**: 1-24, 1990.

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: The role of xanthophyll cycle carotenoids in the protection of photosynthesis. – Trends Plant Sci. **1**: 21-26, 1996a.

Demmig-Adams, B., Adams, W.W., III: Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. – Planta **198**: 460-470, 1996b.

Dodd, I.C., Critchley, C., Woodall, G.S., Stewart, G.R.: Photoinhibition in differently coloured juvenile leaves of *Syzygium* species. – J. exp. Bot. **49**: 1437-1445, 1998.

Foyer, C.H., Noctor, G.: Leaves in the dark see the light. – Science **284**: 5414-5416, 2000.

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

Horton, P., Ruban, A., Walters, R.G.: Regulation of light harvesting in green plants, indication by nonphotochemical quenching of chlorophyll fluorescence. – Plant Physiol. **106**: 415-420, 1994.

Horton, P., Ruban, A.V., Walters, R.G.: Regulation of light harvesting in green plants. – Annu. Rev. Plant Physiol. Plant mol. Biol. **47**: 655-684, 1996.

Ji, B.H., Jiao, D.M.: Photochemical efficiency of PSII and characteristics of photosynthetic CO_2 exchange in *Indic*a and *Japonica* subspecies of rice and their reciprocal cross F1 hybrids under photoinhibitory conditions. – Acta bot. sin. **41**: 508-514, 1999.

Ji, B.H., Jiao, D.M.: Photoinhibition and photoxidation in leaves of *Indica* and *Japonica* rice under different temperatures and light intensities. – Acta bot. sin. **43**: 714-720, 2001.

Ji, B.H., Li, Z.G., Ge, M.Z., Tong, H.Y., Jiao, D.M.: Traits related to photoinhibition of photosynthesis in *Indic*a and *Japonica* subspecies of rice and their reciprocal cross F1 hybrids. – Acta phytophysiol. sin. **20**: 8-16, 1994.

Ji, B.H., Zhu, S.Q., Jiao, D.M.: Traits related to xanthophylls cycle and photosynthetic CO_2 exchange in *Indica-Japonica* hybrid rice under midday strong light. – Chin. J. Rice Sci. **14**: 149-156, 2000.

Jiao, D., Ji, B.: Photoinhibition in *indica* and *japonica* subspecies of rice (*Oryza sativa*) and their reciprocal F1 hybrids. – Aust. J. Plant Physiol. **28**: 299-306, 2001.

Jiao, D.M., Ji, B.H., Tong, H.Y., Li, Z.G.: Photoinhibitory characteristics *Indica-Japonica* subspecies F1 hybrid rice. – Acta bot. sin. **36**: 190-196, 1994.

Khan, M.N.A., Murayama, S., Ishimine, Y., Tsuzuki, E., Nakamura, I.: Physio-morphological studies of F1 hybrids in rice (*Oryza sativa* L). I. Heterosis in characters related to matter production. – Jap. J. Crop Sci. **66** (Extra issue 2): 66-67, 1997.

Khan, M.N.A., Murayama, S., Ishimine, Y., Tsuzuki, E., Nakamura, I.: Physio-morphological studies of F1 hybrids in rice (*Oryza sativa* L). Photosynthetic ability and yield. – Plant Product. Sci. **1**: 233-239, 1998.

Kitajima, M., Butler, W.L.: Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. – Biochim. biophys. Acta **376**: 105-115, 1975.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. – Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Lin, Z.-F., Peng, C.-L., Lin, G.-Z., Ou, Z.-Y., Yang, C.-W., Zhang, J.-L.: Photosynthetic characteristics of two new chlorophyll *b*-less rice mutants. – Photosynthetica **41**: 61-67, 2003a.

Lin, Z.-F., Peng, C.-L., Lin, G.-Z., Zhang, J.-L.: Alteration of components of chlorophyll-protein complexes and distribution of excitation energy between the two photosystems in two new rice chlorophyll *b*-less mutants. – Photosynthetica **41**: 589-595, 2003b.

Long, S.P., Humphries, S., Falkowski, P.G.: Photoinhibition of photosynthesis in nature. – Annu. Rev. Plant Physiol. Plant mol. Biol. **45**: 633-662, 1994.

Mann, C.C.: Crop scientists seek a new revolution. – Science **283**: 310-314, 1999.

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

Murchie, E.H., Chen, Y.Z., Hubbart, S., Peng, S., Horton, P.: Interactions between senescence and leaf orientation determine in situ patterns of photosynthesis and photoinhibition in field-grown rice. – Plant Physiol. **119**: 553-563, 1999.

Niyogi, K.K.: Photoprotection revisited: Genetic and molecular approaches. – Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 333-359, 1999.

Niyogi, K.K.: Safety valves for photosynthesis. – Curr. Opin. Plant Biol. **3**: 455-460, 2000.

Powles, S.B.: Photoinhibition of photosynthesis induced by visible light. – Annu. Rev. Plant Physiol. **35**: 15-44, 1984.

Schreiber, U., Bilger, W.: Rapid assessment of stress effects on plant leaves by chlorophyll fluorescence measurements. – In: Tenhunen, J.D., Catarino, F.M., Lange, O.L., Oechel, W.C. (ed.): Plant Response to Stress – Functional Analysis in Mediterranean Ecosystems. Pp. 27-53. Springer-Verlag, Berlin – Heidelberg – New York – London – Paris – Tokyo 1987.

Wang, Q., Zhang, Q.D., Jiang, G.M., Lu, C.M., Kuang, T.Y.: Photosynthetic characteristics of two super-rice. – Acta bot. sin. **42**: 1285-1288, 2000.

Xu, C.C., Jeon, Y.A., Lee, C.H.: Relative contributions of photochemical and non-photochemical routs to excitation energy dissipation in rice and barley illuminated at a chilling temperature. – Physiol. Plant. **107**: 447-453, 1999.

van Kooten, O., Snel, J.F.H.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. – Photosynth. Res. **25**: 147-150, 1990.