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Leaf chloroplast ultrastructure and photosynthetic properties of a chlorophyll-deficient mutant of rice

Z.M. WU[*], X. ZHANG[**], J.L. WANG[**], and J.M. WAN[**+]

Key Laboratory of Crop Physiology, Ecology and Genetic Breeding, Ministry of Education and Jiangxi Province, Jiangxi Agricultural University, Nanchang 330 045, China[*]
The National Key Facility for Crop Gene Resources and Genetic Improvement, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100 081, China[**]

Abstract

Leaf chloroplast ultrastructure and photosynthetic properties of a natural, yellow-green leaf mutant (*ygl1*) of rice were characterized. Our results showed that chloroplast development was significantly delayed in the mutant leaves compared with the wild-type rice (WT). As leaves matured, more grana stacks formed concurrently with increasing leaf chlorophyll (Chl) content. Except for the lower intercellular CO_2 concentration, the *ygl1* plants had a higher leaf net photosynthetic rate, stomatal conductance, and transpiration rate than those of the WT plants. Under equal amounts of Chl, the excitation energy of PSI and PSII was much stronger in the mutant than that in the WT. The *ygl1* plants showed higher nonphotochemical quenching and lower photochemical quenching. They also exhibited higher actual photochemical efficiency of PSII with a higher electron transport rate. Under the light of 200 μmol(photon) m^{-2} s^{-1}, the *ygl1* mutant showed lesser deepoxidation of violaxanthin in the xanthophyll cycle than WT, but it increased substantially under strong light conditions. In conclusion, the photosynthetic machinery of the *ygl1* remained stable during leaf development. The plants were less sensitive to photoinhibition compared with WT due to the active xanthophyll cycle. The *ygl1* plants were efficient in both light harvesting and conversion of solar energy.

Additional key words: chlorophyll fluorescence; photosynthesis; rice; ultrastructure; violaxanthin.

Introduction

Leaf color mutants are ideal plants to study photosynthesis, biosynthesis, and degradation of Chl, as well as the structure, development, and function of chloroplasts (Terao *et al.* 1985, Zhang *et al.* 2006, Yoo *et al.* 2009, Yu *et al*. 2009). During the early growth stages, leaves of the *ygl1* mutant are yellow. As leaves mature, the color gradually changes to green and fully mature leaves are as dark green as those of the WT (Wu *et al.* 2007). Wu *et al*. (2007) studied the *ygl1* mutant and also conducted position-dependent cloning and functional analysis of the mutated gene. The yellow-green leaf phenotype is controlled by a single recessive nuclear gene in the *ygl1* mutant. The gene encodes for a Chl synthetase (ygl1), which catalyzes the last step of Chl *a* biosynthesis, namely prenylation of chlorophyllide with phytyl diphosphate or geranylgeranyl diphosphate. Chl *a* biosynthesis is the key step for Chl *a* prosthetic protein translation and its accumulation. Thus, it plays a critical role in the assembly of photosynthetic complexes within the thylakoid membrane. The *in vivo* esteration activity of recombinant proteins shows that the esteration activity of the ygl1 protein was lower than that of the WT due to

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[+] Corresponding author; e-mail: wanjm@caas.net.cn
Abbreviations: A – antheraxanthin; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DEPS – deepoxidation ratio; ETR – electron transport rate; FM – fresh mass; F_m − maximal fluorescence in dark-adapted leaves; F_v − maximum variable fluorescence in dark-adapted leaves; g_s – stomatal conductance; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; q_P – photochemical quenching; *E* – transpiration rate; V – violaxanthin; WT – wild-type rice; Φ_{PSII} − actual PSII efficiency; Z – zeaxanthin; β-Car – β-carotene.
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a mutation from proline into serine. The reduced enzymatic activity of Chl synthetase slows down the biosynthesis of Chl and causes massive accumulation of intermediates (Wu *et al*. 2007). However, the ultrastructure and physiological properties have not been studied in the Chl synthetase mutants. In this work, we studied the photosynthetic rate, the low-temperature Chl fluorescence spectra of thylakoid membranes, the xanthophyll cycle, and the chloroplast ultrastructure of the *ygl1* mutant of rice. We aimed to provide an in-depth understanding of the mutant photosynthesis. This plant material could be used in breeding to improve photosynthetic efficiency in rice production.

Materials and methods

Growth: WT and *ygl1* were grown on moist vermiculite in a greenhouse under irradiation of 160 μmol(photon) m^{-2} s^{-1}. To ensure proper irradiation, the greenhouse was supplemented by metal halide and sodium vapor lamp (75 W, *Philips*, China).

Irradiance of 50 and 200 μmol(photon) m^{-2} s^{-1} was provided by a 1,000 W tungsten bulb (*Philips*, China). A water tank with recycled water was used between the radiation source and samples to absorb heat.

Photosynthesis and Chl *a* fluorescence: At the booting stage, 5 uniform plants were selected randomly from the experimental plot. A *CI-340 Handheld Photosynthesis System* (*CID Inc*., USA) was used to measure net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 (C_i), and transpiration rate (E) under natural light conditions. For each plant, the flag leaves at 7-d post fully expansion were chosen and 3 measurements were taken on each leaf. The measurements were taken on a clear day at 9:00 h, when the light intensity was > 1,000 μmol(photon) m^{-2} s^{-1}, and CO_2 concentration 350 μL L^{-1}.

The light-inducible Chl fluorescence kinetics was performed using an *OS5-FL Modulated Fluorometer* (*Opti-Sciences*, Tyngsboro, USA). The minimal fluorescence level (F_0) in the dark-adapted state was measured by the measuring modulated light, which was sufficiently low (<0.1 μmol m^{-2} s^{-1}) not to induce any significant variable fluorescence. To determine the minimal fluorescence level during illumination (F_0'), a black cloth was rapidly placed around the leaf and the leaf-clip holder in the presence of far-red light (7 μmol m^{-2} s^{-1}) in order to oxidize fully the PSII centers. Upon darkening of the leaf, fluorescence dropped to the F_0' level and immediately rose again within several seconds. The maximal fluorescence level in the dark-adapted state (F_m) and the maximal fluorescence level during natural illumination (F_m') were measured by a 0.8-s saturating pulse at 8,000 μmol m^{-2} s^{-1}. F_m was measured after 30 min of dark adaptation. Other parameters were calculated based on measured parameters above (Wang *et al.* 2011). The average of 5 repeated readings was used for each sample.

High-performance liquid chromatography (HPLC): Lutein and β-carotene (β-Car) were extracted using the method of Thayer and Björkman (1990). The samples were extracted in ice-cold 100% acetone and the pigment extracts were filtered through a 0.45 μm membrane filter. The reverse-phase HPLC of pigments was run on a nonendcapped column of *Zorbax ODS* (C-18) with a column length of 250 mm, 4.6 mm of the internal diameter, and a 5-μm particle size (*Zorbax ODS 884950-543*, *Agilent*, California, USA). The analysis was performed on a *LCMS-2010* HPLC mass spectrometry system (*Shimadzu*, Tokyo, Japan) .

Reagents for spectrometry analysis and the preparation of elution buffers were all mass spectrometry grade. Before loading the sample, the column was preequilibrated with 100% methanol for 20 min. After loading the 20 μl of the extract sample, pigments were eluted first using a buffer of acetonitrile:methanol (85:15, v/v) for 14.5 min, then followed by the buffer of acetonitrile:ethyl acetate (68:32, v/v) for 13.5 min, at a flow rate of 1 ml min^{-1}. Finally, the column was equilibrated with a buffer of acetonitrile:methanol (85:15, v/v) for 10 min.

Pigments eluted from the column were monitored using a photodiode array detector, and the eluting peak was monitored by absorbance at 440 nm. The concentration of each pigment in each sample was calculated based on the peak area from the standards of each pigment which was run under the same reverse-phase HPLC condition. The standards of β-Car and lutein were used (*Sigma* Steinheim, Germany).

Low temperature fluorescence emission excitation spectra: Rice seedlings at the 3-leaf stage were used for the preparation of thylakoid membranes following the method of Alfonso *et al*. (1994) with minor modifications. Thylakoids were prepared from leaves homogenized in 0.4 M sorbitol, 100 mM Tricine-KOH (pH 7.5), 10 mM NaCl, and 5 mM $MgCl_2$. After the sample was filtered through 500, 195, and 20 μm nylon mesh and centrifuged for 5 min at 4,000 × *g*, the thylakoid pellet was lysed by resuspending in 5 mM Hepes-KOH, pH 7.5, 10 mM NaCl, and 5 mM $MgCl_2$. The thylakoids were pelleted by centrifugation (5 min, 4,000 × *g*). Finally, thylakoids were washed in 5 mM Hepes-KOH, pH 7.5, 10 mM EDTA, centrifuged, and resuspended in the same buffer with 10% glycerol added. Samples were stored at –80°C in small aliquots. The low temperature (77 K) fluorescence emission excitation spectra of thylakoid membranes were measured using a fluorescence spectrophotometer (*F4500, Hitachi Co.*, Tokyo, Japan). For the analysis, the Chl concentration of the thylakoid

membrane samples was 5 mg L^{-1}. The excitation slit width of the spectrophotometer was set at 10 nm, the emission slit width at 5 nm. The wavelength of excitation light was 436 nm, and the emission light 480 nm. The fluorescence emission of 683 nm (F_{683}) is from the LHC of PSII, and the emission near 730 nm (F_{730}) is produced by the LHC of PSI. The F_{683}/F_{730} ratio is a measure of the excitation energy partition between two photosynthetic reaction centers (Krause and Weis 1991).

Transmission electron microscopy (TEM): Sample preparation for leaf ultrastructure analysis by TEM followed the method of Shan *et al.* (2003). The 2nd top leaves of 1-week-old seedlings and the 1st, 2nd, and 3rd top leaves from 4-week-old seedlings were used. Leaf tips were dissected from plants and fixed for 4 h in a 3% glutaraldehyde fixing solution mixed in a 0.1 mol L^{-1} phosphate buffer (pH 7.2). After washing off the fixative, leaf samples were transferred into 1% osmic acid (pH 7.2) and fixed for 4 h. Samples were then dehydrated in graded acetone of 30, 50, 70, 80, 90, and 100%. The dehydrated samples were embedded in Spurr's resin for 3 d, and allowed to polymerize. The embedded samples were dried by the critical point method after ethanol and amyl acetate dehydration. Ultrathin sections of 1 μm were cut with a microtome (*LKB-V, LKB Company*, Sweden), they were mounted on microscope slides and stained. The chloroplast ultrastructure was examined by *JEM-1230* (*Jeol Ltd*., Tokyo, Japan).

Statistical analysis: For parametric data, an analysis of variance (*ANOVA*) was carried out by *MS Excel 2003*. The *t*-test significance was set at P=0.05 and 0.01 for all tests.

Results

Chloroplast ultrastructure in the *ygl1* mutant: The ultrastructure of leaf chloroplasts was examined in 1- and 4-week-old seedlings of the *ygl1* mutant and the WT (Fig. 1). In the 1-week-old seedlings, the *ygl1* chloroplasts contained only a few stacks of thylakoids, grana were irregularly arranged, and comprised of only 4–6 thylakoids in narrow, curved stripes. Distant lamellae in the grana were loosely arranged. The WT plants formed a large number of stacked grana thylakoids. Compared with the WT chloroplast structure, the chloroplasts in the *ygl1* mutant were not well developed (Fig. 1*A*,*B*). In the 4-week-old, *ygl1* plants, all leaves from the 1st to the 3rd position on the stem had larger grana and thicker grana stacks, but the grana were still arranged irregularly extending radically outward (Fig. 1*D*–*F*).

Photosynthetic properties of *ygl1* mutant: Under natural light conditions, photosynthetic parameters of the *ygl1* mutant were lower than those of the WT plants (Table 1). P_N was lower by 33.8%, g_s by 46.3%, and E by 22.8% in the *ygl1* plants. Conversely, C_i of the *ygl1* mutant was 26.0% higher compared with the WT plants.

Chl fluorescence kinetics of *ygl1* mutant: The maximum photochemical efficiency of PSII (F_v/F_m) and photochemical quenching (q_P) did not differ significantly between the *ygl1* and WT plants (Table 2). The nonphotochemical quenching (NPQ) was 66.67% higher in the *ygl1* mutant than that of the WT plants. Additionally, the actual photochemical efficiency of PSII (Φ_{PSII}) and electron transport rate (ETR) of the *ygl1* plants exceeded the WT by 12.8% and 23.3%, respectively.

Low-temperature (77 K) fluorescence of thylakoid membranes from *ygl1* mutant: Under low temperature, the size of the antennae and the transfer of excitation energy from PSII to PSI affected the fluorescence yield from the both photosystem centers. At the seedling stage, thylakoid membranes from the *ygl1* mutant leaves and WT plants produced two emission peaks of F_{683} and F_{731}, respectively (Fig. 2). Additionally, the peak volume (683 and 730 nm) of the *ygl1* mutant was higher than that of the WT plants. It indicated that the excitation energy in PSI and PSII was higher in the *ygl1* mutant than that in the WT plants with the same Chl concentration.

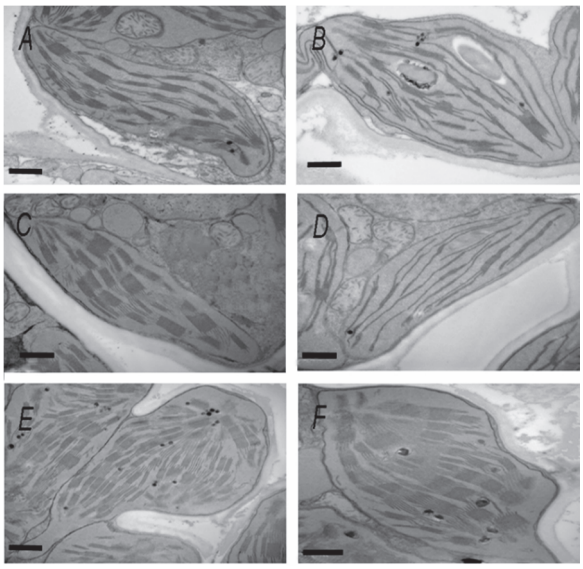

Fig. 1. Transmission electron micrograph of chloroplasts from the *ygl1* mutant and the wild-type rice (WT). Bars 500 nm. *A*: Chloroplasts of the 2nd leaf from the top of 1-week-old WT seedlings. *B*: Chloroplasts of the 2nd leaf from the top of 1-week-old seedlings of *ygl1* mutant. *C*: Chloroplasts of the 1st leaf from the top of 4-week-old seedlings of WT. *D*: Chloroplasts of the 1st leaf from the top of 4-week-old seedlings of *ygl1* mutant. *E*: Chloroplasts of the 2nd leaf from the top of 4-week-old seedlings of *ygl1* mutant. *F*: Chloroplasts of the 3rd leaf from top of 4-week-old seedlings of *ygl1* mutant.

Table 1. Photosynthetic properties of leaves from the *ygl1* mutant and the wild-type rice (WT). ** and – **t-test* significance at 0.01 and 0.05 levels, respectively. Means ± SD, n = 5. P_N – net photosynthetic rate; g_s – stomatal conductance; C_i – intercellular CO_2 concentration; E – transpiration rate.

Plant type	P_N [μmol(CO_2) m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	C_i [μmol(CO_2) mol^{-1}]	E [mol(H_2O) m^{-2} s^{-1}]
WT	26.30 ± 0.51	0.41 ± 0.03	201.63 ± 5.69	9.48 ± 0.19
ygl1	17.40 ± 0.47**	0.22 ± 0.01**	254.25 ± 6.78**	7.32 ± 0.22**

Table 2. Chlorophyll fluorescence parameters of seedlings from the *ygl1* mutant and the wild type (WT) of rice.**,* *t-test* significance at 0.01 and 0.05 levels, respectively. Means ± SD, n = 5. F_v/F_m – maximum photochemical efficiency of PSII; q_P – photochemical quenching; NPQ – nonphotochemical quenching; Φ_{PSII} – actual PSII efficiency; ETR – electron transport rate.

Parameters	WT	*ygl1*
F_v/F_m	0.86 ± 0.03	0.85 ± 0.01
q_P	0.85 ± 0.03	0.83 ± 0.02
NPQ	0.57 ± 0.04	0.94 ± 0.11**
Φ_{PSII}	0.50 ± 0.01	0.57 ± 0.04
ETR	10.50 ± 0.28	12.95 ± 0.92**

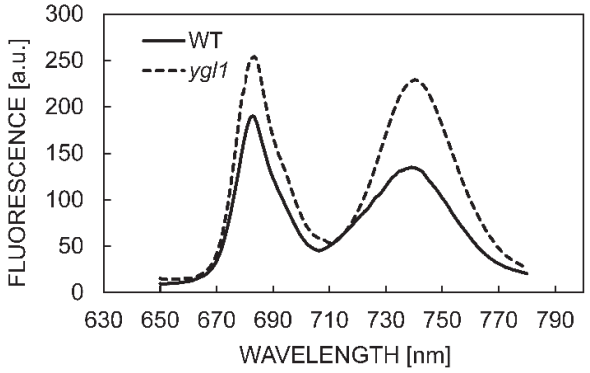

Fig. 2. The low-temperature (77K) fluorescence spectra of thylakoid membrane in the *ygl1* mutant and wild-type (WT) seedlings.

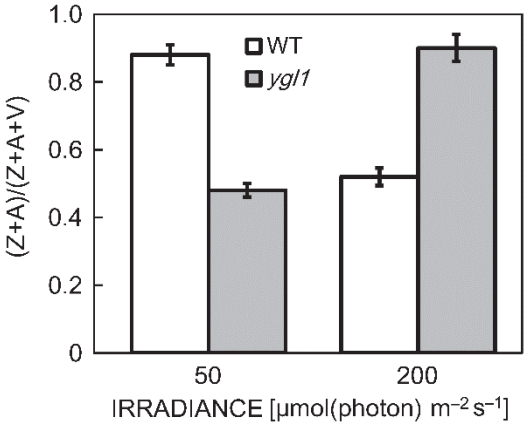

Fig. 3. Ratio of (Z+A)/(Z+A+V) and contents of pigments in the zeaxanthin cycle of leaves from the wild type and the *ygl1* mutant seedling of rice at 50 and 200 μmol (photon) m^{-2} s^{-1}. Z – zeaxanthin; A – antheraxanthin; V – violaxanthin.

The xanthophyll cycle in leaves from *ygl1* seedlings: We found higher contents of zeaxanthin (Z), antheraxanthin (A), and violaxanthin (V) in the WT leaves compared with those of the *ygl1* mutant (Fig. 3). Under light intensity of 50 μmol m^{-2} s^{-1}, the DEPS ratio was higher in the WT leaves than that of the *ygl1* mutant, *i.e.*, 0.88 and 0.48, respectively. However, the *ygl1* mutant had higher ratio (*i.e.*, 0.90) than the WT (0.52) under strong light conditions (Fig. 3). This indicated that the *ygl1* mutant was more tolerant to the photoinhibition than the WT.

Discussion

Chl-deficient mutants are valuable not only as a tool for photosynthetic studies, but also as markers of heterosis (Falbel and Staehelin 1996; Havaux and Tardy 1999). In rice, more than 10 different types of Chl-deficient mutants involving about 80 gene loci have been reported. About 20 Chl-deficient mutations were already mapped to various chromosomes by classical or molecular means (Chi *et al.* 2010) since the *ygl1* mutant was confirmed as affecting Chl content without influencing the plant development. Thus, the trait of yellowish-green leaves could be used as a marker for hybrid rice production.

Photosynthesis is a process in which solar energy absorption, transfer, and conversion are all carried out on the thylakoid membrane of chloroplasts. In higher plants, thylakoid membranes are regularly arranged and stacked into grana in chloroplasts. The presence of grana stacks indicates that the highly compact light harvesting machinery is extremely efficient in the absorption of light quantum and conversion of light energy. Therefore, all mutants in leaf color showed some alteration in structural properties of thylakoid membranes (Zhang *et al*. 2006, Yoo *et al*. 2009). In the *ygl1* mutant, chloroplasts contained fewer grana stacks, fewer thylakoid lamellae per granum, and the grana were arranged irregularly. These are the signs of underdeveloped chloroplast ultrastructure (Schmid 2008, Cazzonelli and Pogson 2010). But as the leaves matured, the number of grana increased, which would be beneficial for maintaining a stable photosynthetic structure. Such structural features of chloroplasts matched with the physiological properties of the mutant,

and it delayed the leaf greening process, because leaves accumulated Chl very slowly.

The fluorescence kinetics parameter, F_v/F_m, is widely used for indicating the primary maximum photochemical efficiency or the potential photochemical efficiency of PSII. It is often considered the index of physiological stress, as it can reflect the physiological status of photosynthetic organelles in intact plants. Factors contributing to the inhibition of PSII cause a decrease in the F_v/F_m ratios. NPQ has been recognized as protecting mechanism for the photochemical reaction center from light injury; it plays a key role in regulating the photochemical efficiency of PSII.

When comparing the *ygl1* seedlings with the WT ones, no significant difference in the F_v/F_m ratios were found. However, the mutant showed significantly higher NPQ (by 66.7%) than the WT plants. These results suggested that the mutant was more effective in dissipation and consumption of the absorbed light energy than the WT. Despite the reduced q_P (by 2.7%), the mutant plants manifested still 12.8% higher Φ_{PSII} and 23.3% higher ETR than the WT (Table 2). According to this, the mutant leaves had substantially higher photosynthetic efficiency.

To maintain a high photosynthetic rate, the excitation energy must be balanced between PSII and PSI (Zhang 1988, Tang *et al.* 2005). With equal Chl content, the F_{683}/F_{730} ratio was 1.11 ± 0.06 in the mutant, which was 21.28% lower than that of the WT (1.41 ± 0.08). The lower F_{683}/F_{730} ratio indicated that the excitation energy was transferred more efficiently to the PSI center in the *ygl1* mutant.

The xanthophyll cycle is the process in which V is converted by violaxanthin de-epoxidase into A under strong light conditions. It is considered as the nonphotochemical, quenching pathway. The final step converting the intermediate, A, to Z is catalyzed by zeaxanthin epoxidase. Under low light conditions, the reactions proceed in the reverse direction; Z is converted back to V. DEPS, the ratio of (Z+A)/(Z+A+V), indicates the de-epoxidation level. Under stress conditions, such as strong light, drought, high temperature, and aging, the xanthophyll cycle is activated to protect the photosystem from overexcitation, maintain electron transfer efficiency, and protect the lipids in the thylakoid membrane from photo-oxidative damage (Havaux and Tardy 1999). Photoinhibition is a limiting factor for a high and stable rice yield. A cultivar with high yield potential should be able to adapt to diverse environments, especially, to various light intensities (Preiss *et al*. 1995, Chen *et al*. 2008). Under light intensity of 50 μmol(photon) m^{-2} s^{-1}, the WT leaves showed the higher DEPS compared with the *ygl1* mutant, while it was reverse under 200 μmol m^{-2} s^{-1} (Fig. 3). Thus, we concluded that the *ygl1* mutant plants should tolerate better the photoinhibitory effect of stronger light than the WT (Demmig-Adams and Adams 1992).

References

Alfonso, M., Montoya, G., Cases, R. *et al*.: Core antenna complexes, CP43 and CP47, of high plant photosystem II spectral properties, pigment stoichiometry, and amino acid composition. – Biochemistry **33**: 10494-10500, 1994.

Cazzonelli, C.I., Pogson, B.J.: Source to sink, regulation of carotenoid biosynthesis in plants. – Trends Plant Sci. **15**: 266-274, 2010.

Chen, Q., Wang, L.F., Su, N., *et al*.: Photosystem 2 photochemistry and pigment composition of a yellow mutant of rice (*Oryza sativa* L.*)* under different irradiances. – Photosynthetica **46**: 35-39, 2008.

Chi, X.-F., Zhou, X.-S., Shu,Q.-Y.: Fine mapping of a *Xantha* mutation in rice (*Oryza sativa* L.). – Euphytica **172**: 215-220, 2010.

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

Falbel, T.G., Staehelin, L.A.: Partial block in the early steps of the chlorophyll synthesis pathway: A common feature of chlorophyll *b*-deficient mutants. – Physiol. Plantarum **97**: 311-320, 1996.

Havaux, M., Tardy, F: Loss of chlorophyll with limited reduction of photosynthesis as an adaptive response of Syrian barley landraces to high-light and heat stress. – Aust. J. Plant Physiol. **26**: 569-578, 1999.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis. – Annu. Rev. Plant Phys. **42**: 313-349, 1991.

Preiss, S., Thornber, J. P.: Stability of the apoproteins of light-harvesting complex I and II during biogenesis of thylakoids in the chlorophyll *b*-less barley mutant *chlorina f2*. – Plant Physiol. **107**: 709-717, 1995.

Schmid, V.H.R.: Light-harvesting complexes of vascular plants. – Cell. Mol. Life Sci. **65**: 3619-3639, 2008.

Shan, X.Q., Wang, H.O., Zhang, S.Z. *et al*.: Accumulation and uptake of light rare earth elements in a hyperaccumulator *Dicropteris dichotoma.* – Plant Sci. **165**: 1343-1353, 2003.

Tang, Y.L., Wen, X.G., Lu, C.M.: Differential changes in degradation of chlorophyll–protein complexes of photosystem I and photosystem II during flag leaf senescence of rice. – Plant Physiol. Bioch. **43**: 193-201, 2005.

Terao, T., Yamashita, A., Katoh, S.: Chlorophyll *b*-deficient mutants of rice. I. Absorption and fluorescence spectra and chlorophyll *a*/*b* ratios. – Plant Cell Physiol. **26**: 1361-1367, 1985.

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

Wang, L.F., Chen, Y.Y.: Photosynthetic characterization at different senescence stages in and early senescence mutant of rice *Oryza sativa* L. – Photosynthetica **49**: 140-144, 2011.

Wu, Z.M., Zhang, X., He, B. *et al*.: A chlorophyll-deficient rice mutant with impaired chlorophyllide esterification in chlorophyll biosynthesis. – Plant Physiol. **145**: 29-40, 2007.

Yamamoto, H.Y.: Xanthophyll cycles. – Methods Enzymol. **110**: 303-312, 1985.

Yoo, S.C., Cho, S.H., Sugimoto,H., *et al*.: Rice *Virescent3* and

Stripe1 encoding the large and small subunits of ribonucleotide reductase are required for chloroplast biogenesis during early leaf development. – Plant Physiol. **150**: 388-401, 2009.

Yu, Q.B., Jiang,Y., Chong, K., Yang, Z.N.: *AtECB2,* a pentatricopeptide repeat protein, is required for chloroplast transcript *accD* RNA editing and early chloroplast biogenesis in *Arabidopsis thaliana*. – Plant J. **59**: 1011-1023, 2009.

Zhang, Q.D.: Effects of additional factors on fluorescent characteristics of chloroplasts from wheat seedlings grown under different light intensities. – Acta Bioph. Sin. **4**: 192-196, 1988.

Zhang, H.T., Li, J.J., Yoo, J.H. *et al*.: Rice *chlorina-1* and *chlorina-9* encode ChlD and ChlI subunits of Mg-chelatase, a key enzyme for chlorophyll synthesis and chloroplast development. – Plant Mol. Biol. **62**: 325-337, 2006.