BIOLOGIA PLANTARUM 39 (2): 251-256, 1997

Low irradiance stress tolerance in rice (*Oryza sativa* L.)

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Abstract

The effect of low irradiance on three rice cultivars (shade tolerant cvs. Swarnaprabha and CO 43 and shade susceptible cv. IR 20) was studied. The large subunit (LSU) of ribulose-1,5-bisphosphate carboxylase/oxygenase with molecular mass of 55 kDa was reduced in cv. IR 20 grown under low irradiance (LI). Native protein profile studied showed, under LI, reduction in the contents of proteins with R_F values 0.03, 0.11 and 0.37. Analysis of chloroplast polypeptides revealed an induction of light-harvesting chlorophyll-protein 2 (LHCP2) under shade. The induction was more expressed in cv. CO 43 than in cv. IR 20. Under LI, *in vivo* labelled protein bands in the molecular range of 26 - 27 kDa were induced. These proteins were highly turned over in the LI-grown plants of cv. CO 43 than in cv. IR 20. A signal for *rbcL* gene sequences in EcoRI digested lanes was also found. Isozyme analysis of peroxidase showed an induction of a new band with R_F 0.43 in cv. IR 20 subjected to LI.

Additional key words: chloroplast proteins, light-harvesting chlorophyll-protein 2, peroxidase, *rbcL* gene sequences, SDS-PAGE.

Introduction

In India as well as in south east Asian countries, 80 % of rice is grown during the monsoon season, when irradiance is 40 to 60 % less than that of dry season. Yield reduction of more than 50 % has been reported under low irradiance (LI) (Venkateswarlu 1977). The susceptibility of high yielding rice cultivars to LI makes them inefficient yielders. Efforts made so far to develop suitable management technologies or tolerant cultivars have not yielded desirable results due to lack of proper understanding of tolerance mechanisms. Hence, investigations into the molecular basis for tolerance to LI will help to isolate cultivars efficient under LI. Attempts were made in this study to elicit information on the aspects of protein profile change, chloroplast gene expression and peroxidase pattern changes occurring in the LI tolerant and susceptible cultivars under LI.

Received 22 February 1996, *accepted* 1 May 1996.
Acknowledgement: The financial support extended by the Council of Scientific and Industrial Research, New Delhi (India) to the senior author is gratefully acknowledged.

Materials and methods

Rice (*Oryza sativa* L.) cultivars Swarnaprabha, CO 43 and IR 20 were grown under LI (about 50 % of normal) by using white gada cloth. The ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) protein profile was studied by electrophoretic separation on SDS-PAGE (Laemmli 1970). Samples of 500 mg leaf tissues were homogenized with 1.0 cm^3 of the isolation buffer at 4 °C [5.0 mM $MgCl_2$, 50 mM HEPES (free acid), 5.0 mM EDTA, 5.0 mM $NaHCO_3$, and 40mM mercapto-ethanol] and centrifuged at 167 rps for 10 min at 4 °C. The clear supernatant was used for the analysis of RuBPCO.

Native proteins were studied by nondenaturing gel electrophoresis (Laemmli 1970). Proteins were extracted in phosphate buffer (pH 7.0) and centrifuged at 18 000 *g* for 15 min. The supernatant was used for the study.

Chloroplast proteins were analysed by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). Intact chloroplasts were isolated by the method of Brook and Milligan (1989) and then, the chloroplast suspension was mixed with 10 % ice cold trichloroacetic acid (TCA) and left on ice for 10 min. The solution was centrifuged at 3 000 *g* for 5 min. Traces of TCA and pigments were removed by washing thrice with ice cold acetone. The final pellet was washed with cold diethyl ether and dried to a powder. The powdery protein sample was thoroughly solubilized in a minimal volume of 2.0 % SDS. A known aliquot of the sample was used to estimate the protein content and the protein analysis was carried out by SDS-PAGE by the method of Laemmli (1970).

To identify the *in vivo* labelled leaf proteins, leaves of about 500 mg were cut into 5.0 mm^2 segments and placed in glass tubes containing 5.0 cm^3 of 20 mM Tris-HCl buffer (pH 7.5). The tubes with samples were kept in a thermostated water bath at 25 °C under normal irradiation for 60 min. The 1.85 MBq of ^{35}S-methionine was added and labelling continued for 2 h under shaded and normal irradiation for the treated and control samples, respectively. At the end of the labelling, the leaf segments were washed with 1.0 mM methionine (cold). The protein extraction and analysis by SDS-PAGE were carried out and the gel was dried and subjected to autoradiography.

The chloroplast gene expression was tested by isolating the chloroplast DNA by the method of Brook and Milligan (1989). The isolated chloroplast DNA was subjected to restriction digestion using the restriction enzymes *EcoRI*, *Bam H I* and *Sal I* (*Grenei Pvt.*, Bangalore, India). Hybridization was carried out using *rbcL* probe. The method of Sadasivam and Manickam (1992) was followed to study the peroxidase isozyme patterns.

Results and discussion

The large subunit of RuBPCO that contains the active site is encoded by the gene *rbcL* in the chloroplast genome. The isolated RuBPCO protein of the tested rice cultivars was resolved on 10 % SDS-PAGE and stained in Coomassie brilliant blue

(Fig. 1). The molecular mass (M_r) of the polypeptide bands ranged from 14 to 70 kDa. The major polypeptide band with M_r of 55 kDa corresponded to the large subunit of RuBPCO. The intensity of this band was reduced to a greater extent under LI in the cv. IR 20 and this may be the reason for the susceptible nature of IR 20 to LI. The results obtained in the present study confirm the findings of Yan *et al.* (1992), Sage *et al.* (1993), Sage and Seemann (1993) and Li *et al.* (1992). The LSU of RuBPCO is the major product of chloroplast DNA (Mullet 1988).

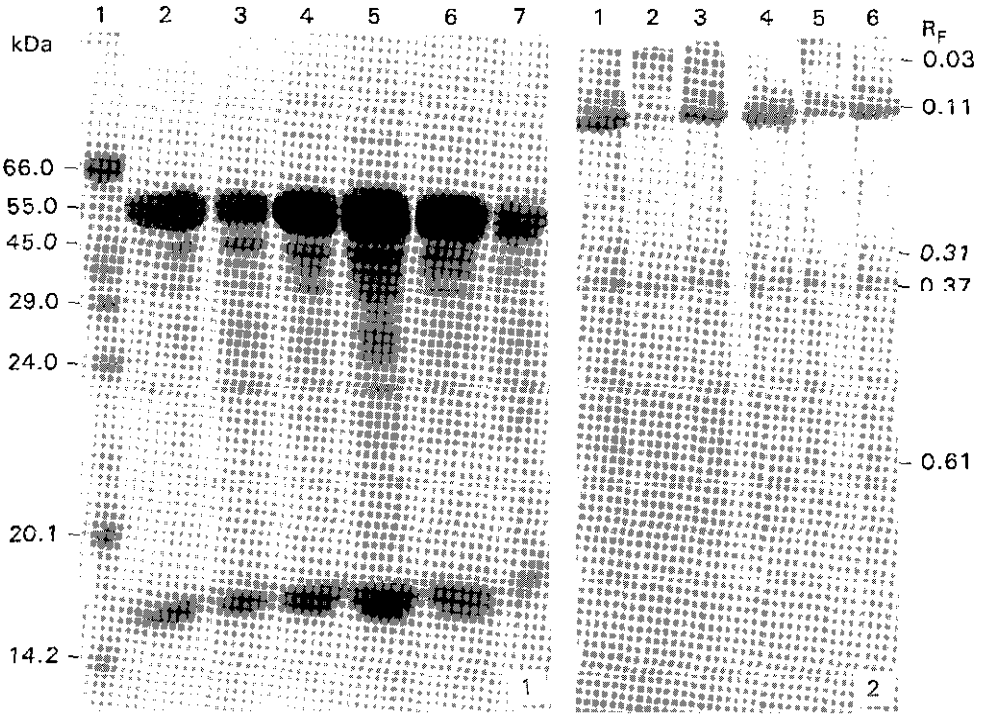

Fig. 1. Influence of low irradiance (lanes 3, 4, 7) on RuBPCO protein profiles of rice cultivars CO 43 (2, 3), Swarnaprabha (4, 5) and IR 20 (6, 7). *1 - markers.*

Fig. 2. Influence of low irradiance (lanes 2, 3, 5) on native proteins of rice cultivars IR 20 (1, 2), Swarnaprabha (3, 4) and CO 43 (5, 6).

The native proteins run on 7.0 % polyacrylamide gels in their native state without any denaturation caused by SDS resolved nine polypeptide bands with R_F values ranging from 0.03 to 0.73. The protein bands with R_F values 0.03, 0.11 and 0.37 were reduced under LI. Nevertheless, the intensities of these bands were relatively higher in the shade-grown plants of Swarnaprabha and CO 43 than in IR 20 (Fig. 2) Subjecting the rice seedlings to LI from 15 d after sowing (DAS) till 45 DAS induced in the cv. Swarnaprabha and cv. CO 43 relatively higher synthesis of LHCP2 with M_r of 25 - 27 kDa as compared to the control plants. Such induction of LHCP2 was not observed in LI-grown IR 20 plants. It is suggested that the high amount of

LHCP2 in cvs. Swarnaprabha and CO 43 helped the plants to absorb and utilize more radiant energy even under LI which lead to higher photosynthetic efficiency and higher yield than in the LI-susceptible cultivar (Fig. 3).

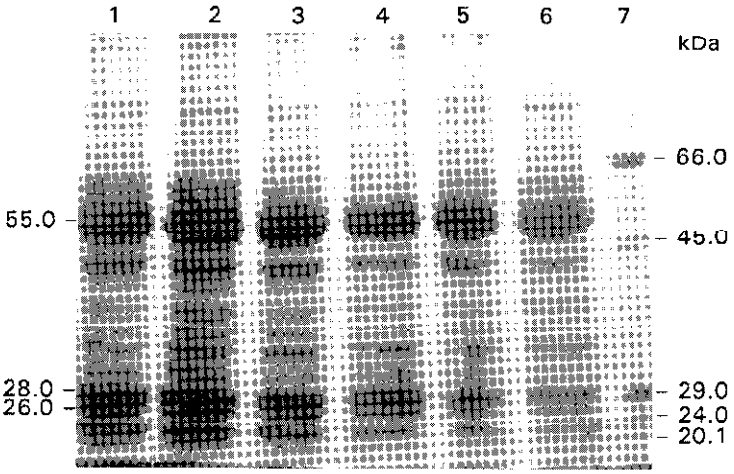

Fig. 3. Influence of low irradiance (lanes 1, 4, 6) on chloroplast proteins of rice cultivars CO 43 (1, 2), Swarnaprabha (3, 4) and IR 20 (5, 6). 7 - markers.

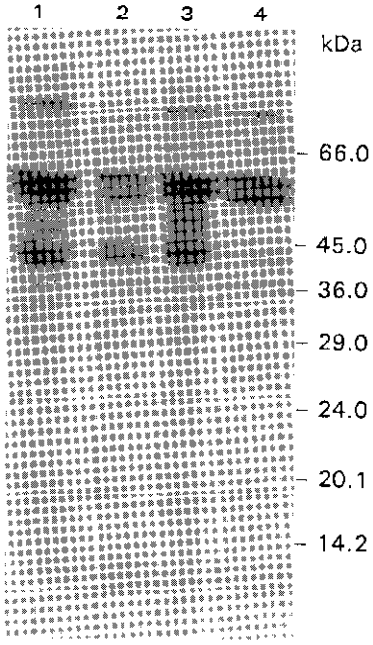

Fig. 4. Influence of low irradiance (lanes 2, 3) on *in vivo* protein synthesis by leaf segments from rice cultivars CO 43 (1, 3) and IR 20 (2, 4).

Polypeptides associated with LHCP are encoded by nuclear *cab* genes. According to Peter and Thornber (1988), the LHCP2 consists of at least six separable oligomeric components with M_r of 25 - 27 kDa. The induction of these proteins under LI in the present study indicated that the expression of the respective genes might be involved in the ability of plants to tolerate the LI stress (Fig. 4).

Apart from these proteins, the intensity of the bands with M_r of 76, 60 and 35 kDa was influenced by the LI treatment: in CO 43 it was high whereas in IR 20 it was much reduced. Thus at LI, the tolerant cultivar induced or maintained the synthesis of proteins with M_r of 25-27, 55, 76 and 35 kDa. The induced proteins probably protect against both structural and functional damages caused by LI.

Good quality and quantity of chloroplast DNA was obtained by the procedure of Brook and Milligan (1989) using hexadecyl trimethyl ammonium bromide (CTAB). The DNA was restricted using the restriction endonucleases namely *EcoRI*, *Bam HI* and *Sal I*. Lambda DNA digested with *Hind III* was used as a marker and the DNA was Southern-transferred to a nitrocellulose membrane. The photosynthetic probe *PTB 29* (*rbcL*) was nick translated using ^{32}P-labelled *dCTP*. The immobilized DNA on the nitrocellulose membrane was hybridized with the radiolabelled denatured *rbcL*

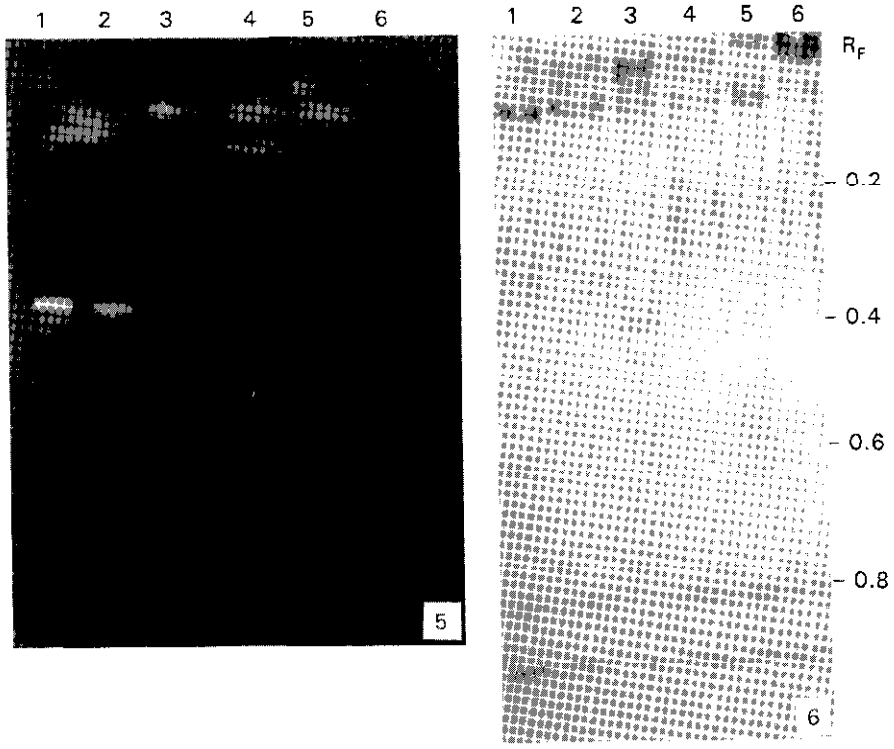

Fig. 5. Autoradiogram showing chloroplast DNA hybridization in rice cultivars CO 43 (lanes 1, 3, 5) and IR 20 (2, 4, 6). Digestion with *EcoRI* (1, 2), *Bam HI* (3, 4) or *Sal I* (5, 6).

Fig. 6. Influence of low irradiance (lanes 1, 3, 5) on the peroxidase band pattern in rice cultivars CO 43 (1, 2), IR 20 (3, 4) and Swarnaprabha (5, 6).

probe and the autoradiogram developed showed signals for *rbcL* (LSU of RuBPCO) gene sequences in *EcoRI* digested lanes. From this experiment, the sequences of *rbcL* (LSU) were localized (Fig. 5).

Isozymes are ideal markers for biotic and abiotic stress tolerance. Electrophoretic patterns of isozymes represent a valid tool in detecting genetic and epigenetic variations (Allicchio *et al.* 1987). The peroxidase isozyme system has been widely used in genetic studies in higher plants because it supplies high number of genetic markers (Rebordinos and Perez 1987). In the present study, the peroxidase was characterized by the presence of two zones, a fast migrating zone and a relatively slow migrating zone (Fig. 6). Thus in the LI-treated IR 20 plants, three bands appeared in the slow migrating zone, while in the other two cultivars only two bands corresponding to R_F values of 0.03 and 0.10 appeared under both control and LI conditions.

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