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Differential photosynthetic acclimation pattern to limiting growth-irradiance in two types of C_4 plants

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Abstract

Photosynthetic acclimation to reduced growth irradiances (650 and 200 μmol m^{-2} s^{-1}) in *Eleusine coracana* (L.) Garten, a nicotinamide adenine dinucleotide-malic enzyme (NAD-ME) C_4 species and *Gomphrena globosa* L., a nicotinamide adenine dinucleotide phosphate-malic enzyme (NADP-ME) C_4 species were investigated. *E. coracana* plants acclimated in 4 and 8 d to 650 and 200 μmol m^{-2} s^{-1}, respectively, whereas *G. globosa* plants took 8 and 10 d, respectively, to acclimate to the same irradiances. The acclimation to reduced irradiance was achieved in both species by greater partitioning of chlorophyll towards the light-harvesting antennae at the expense of functional components. However, magnitude of increase in the light-harvesting antenna was higher in *E. coracana* as compared to *G. globosa.* Superior photosynthetic acclimation to reduced irradiance in *G. globosa* was due to the smaller change in functions of the cytochrome b_6/f complex, photosystem (PS) 1 and PS2 leading to the higher carbon fixation rates compared to *E. coracana.*

Additional key words: chlorophyll amount and fluorescence; cytochrome b_6/f; electron transport rates; net photosynthetic rate; photosystems 1 and 2; quantum efficiency of photosystem 2.

Introduction

Plants acclimated to high irradiance develop alterations at the molecular level when exposed to reduced growth irradiance (Anderson and Andersson 1988). They acclimate by changing their thylakoid membrane composition, the light-harvesting complex (LHC2), chloroplast ultrastructure, and conductances for gas exchange (Björkman 1973, Boardman 1977, Lichtenthaler 1981, Lichtenthaler *et al.* 1981, Melis and Harvey 1981, Rao and Rama Das 1982, Hodges and Barber 1983, Leong and Anderson 1984, Lichtenthaler and Meier 1984, Bhaskar and Rama Das 1987, Chow and Hope 1987, Burkey and Wells 1991, Evans 1993, Koesmaryono *et al.* 1998). However, there have been some exceptions (Lee and Whitmarsh 1989, Chow *et al.* 1991, McKiernan and Baker 1991, Burkey and Wells 1996). The time course of acclimation has been studied in several C_3 plants, *e.g., Solanum, Pisum, Lycopersicon, Phaseolus, Hordeum* (Caemmerer and Farquhar 1984, Davies *et al.* 1986, Ferrar and Osmond 1986, Chow and Anderson 1987, De la Torre and Burkey 1990) and one NAD-ME C_4 dicot species, *Amaranthus hypochondriacus* (Sailaja and Rama Das 1995a). The present study is a comparison of the behaviour of other C_4 species, *Eleusine coracana* (an NAD-ME C_4) and *Gomphrena globosa* (an NADP-ME C_4) to reduced growth irradiance. In an earlier study we observed that down-regulation of C_4 metabolism was accomplished in identical time periods in plants of different C_4 metabolising types, rate-limiting steps being the decarboxylating enzyme(s) of the pathways (Sailaja and Rama Das 1995b). Therefore, the present major objective was to identify species specific characters, if any, between the two plant species by studying the modulation of thylakoid membrane composition and function.

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Abbreviations: Asc - ascorbate; BQ - benzoquinone; Chl - chlorophyll; Cyt – cytochrome; DCPIP - dichlorophenol indophenol; DCMU - 3(3,4-(dichlorophenyl)-1,1-dimethyl urea; F_0 - fluorescence when reaction centres are open; F_v - variable fluorescence; F_m - maximum fluorescence; F_{690} – florescence at 690 nm measured at 77 K; F_{735} - fluorescence at 735 nm measured at 77 K; HEPES - N-2-hydroxyethyl piperazine N-2-ethanesulfonic acid; MV – methyl viologen; HI - high irradiance; LI - low irradiance; NAD-ME – NAD-malic enzyme; NADP-ME – NADP-malic enzyme; P_N – net photosynthetic rate; PS - photosystem.

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Materials and methods

Plant growth: *Eleusine coracana* (L.) Gaertn. and *Gomphrena globosa* L. plants that were raised from seed in the open field under natural conditions receiving full sunlight (2000 μmol m^{-2} s^{-1}) for 45 d, were designated as high irradiance (HI) plants. Two other batches of plants were grown at one-third (650 μmol m^{-2} s^{-1}) and one tenth of this irradiance (200 μmol m^{-2} s^{-1}) and designated LI_1 and LI_2 plants, respectively. Reduced irradiances were obtained by screening full sunlight through appropriate wooden meshes. The photoperiod was approximately 12 h. For studying the time course of acclimation, HI plants were transferred to reduced irradiances (650 μmol m^{-2} s^{-1} and 200 μmol m^{-2} s^{-1}) while LI_1 and LI_2 plants were transferred to full irradiance. Plants grown under HI transferred to LI were called HI→LI_1 and HI→LI_2, respectively, and those grown under LI transferred to HI were called LI_1→HI and LI_2→HI, respectively. The time course was measured with respect to controls on every day following transfer. The day temperature varied from 30 to 40 °C. Night temperature varied from 12 to 25 °C. Experiments were performed from October 1992 to May 1993. Hyderabad is situated at 17°10'N latitude and 78°23'E longitude at an altitude of 542.6 m above mean sea level. The values are means ± SD of ten independent measurements per trial and three such trials. Statistics was done using *t*-test.

Chlorophyll (Chl) content was estimated spectrophotometrically according to the procedure of Arnon (1949).

Chl fluorescence emission spectra of thylakoid membranes were recorded at 490 nm excitation using a fluorescence spectrophotometer (*Hitachi* model *3010*) with a liquid N_2 attachment. Chl fluorescence transients at 77 K were measured according to the procedure of Powles and Björkman (1982) using the same fluorescence spectrophotometer. Chl fluorescence transients in the field were measured using a portable Chl fluorimeter (model *SF-30*, *Richard Branker*, Canada).

Thylakoid membranes were isolated according to the procedure of Vainstein *et al.* (1989).

Photosynthetic electron transport rates: Whole chain electron transport rates (H_2O→MV) and PS1 (DCPIP→MV) were measured polarographically at 25 °C as O_2 uptake using Clark-type electrode in *Gilson Oxygraph* according to the procedure of De la Torre and Burkey (1990). PS2 activity was measured as DCPIP reduction at 590 nm using molar extinction coefficient for DCPIP which is 18.9 mM^{-1} cm^{-1}.

Cytochrome (Cyt) *f* was determined according to the procedure of Bendall *et al.* (1971) in a dual-beam dual-wavelength spectrophotometer (*Hitachi* model *557*). The Cyt *f* concentration was calculated using molar extinction coefficient of 19.7 mM^{-1} cm^{-1}.

Net photosynthetic rate (P_N) was measured using open gas exchange system (*ADC* portable IRGA comprising *LCA-2* analysers) in differential mode.

Results

The effect of reduced irradiance on the ratios Chl *a/b*, F_{690}/F_{735} at 77 K, and Chl/Cyt *f* was studied to see if the light-harvesting antenna changed under these conditions. The values of the Chl *a/b* ratios decreased in LI_1 and LI_2 plants of *E. coracana* and *G. globosa* compared to HI plants (Fig. 1). HI→LI_1 and HI→LI_2 plants of both species showed a reduction in Chl *a/b* ratios while increases in the ratios were observed in LI_1→HI and LI_2→HI plants (Fig. 1). The time taken for HI→LI_1 and LI_1→HI plants to show changes in Chl *a/b* ratio in *E. coracana* was 4 d whereas it was 8 d in *G. globosa*. The time course of acclimation was 8 d in HI→LI_2 and LI_2→HI plants of *E. coracana* while it was 10 d for similar *G. globosa* plants (Fig. 1). Fluorescence spectra at 77 K in both species showed fluorescence emission peaks at 690 and 735 nm. The LI_1 and LI_2 plants of both *E. coracana* and *G. globosa* showed an increase in the F_{690}/F_{735} ratio at 77 K compared to the HI plants (Table 1). The F_{690}/F_{735} ratio increased in HI→LI_1 and HI→LI_2 plants of both *E. coracana* and *G. globosa* while it decreased in LI_1→HI and LI_2→HI plants of both species. However, the magnitude of change was larger in *E. coracana* compared to that of *G. globosa*. The time period of acclimation was identical for all parameters studied to that observed for the Chl *a/b* ratio. Therefore, the variation is presented for the last day of acclimation in the table. As expected, the Chl/Cyt *f* ratio increased in HI→LI_1 and HI→LI_2 plants of both *E. coracana* and *G. globosa* while the ratio decreased in LI_1→HI and LI_2→HI plants of both species. The magnitude of change in the ratio in *G. globosa* was not as great as in *E. coracana*.

The values of the variable Chl fluorescence ratio F_v/F_m were measured in order to assess the effect of reduced irradiance on function of PS2. The values at field temperature were typically above 0.33±0.06 which is in accordance with the measurements of Sthapit *et al.* (1995) and Sailaja and Rama Das (1996b) on rice cultivars and diaheliotropic plants, respectively, by using

a similar *SF 30* fluorimeter. After correcting F_0, the mean F_v/F_m was 0.78±0.04 at field temperature in HI plants (Table 1). The determination of F_0 is problematic in non-modulated fluorescence induction at room temperatures especially if the exciting radiation is switched on slowly. An apparent F_0 measured after the shutter opening may contain a contribution of F_v (Tyystjärvi and Karunen 1990). Making a correction to F_0 according to the procedure of Morissette and Popovic (1987) solved this problem. The F_v/F_m ratio was low in LI_1 and LI_2 plants of *E. coracana* and *G. globosa*. The ratio decreased in HI→LI_1 and HI→LI_2 and increased in LI_1→HI and LI_2→HI plants for both *E. coracana* and *G. globosa*. The decline in F_v/F_m was contributed from both increase in F_0 and decrease in F_m rather than decrease in both F_0 and F_m (values not presented). The effect of reduced irradiance on F_v/F_m at field temperature was higher than the effect seen at 77 K. The effect was more pronounced in *E. coracana* plants acclimated to reduced growth-irradiance compared to the similar plants of *G. globosa*.

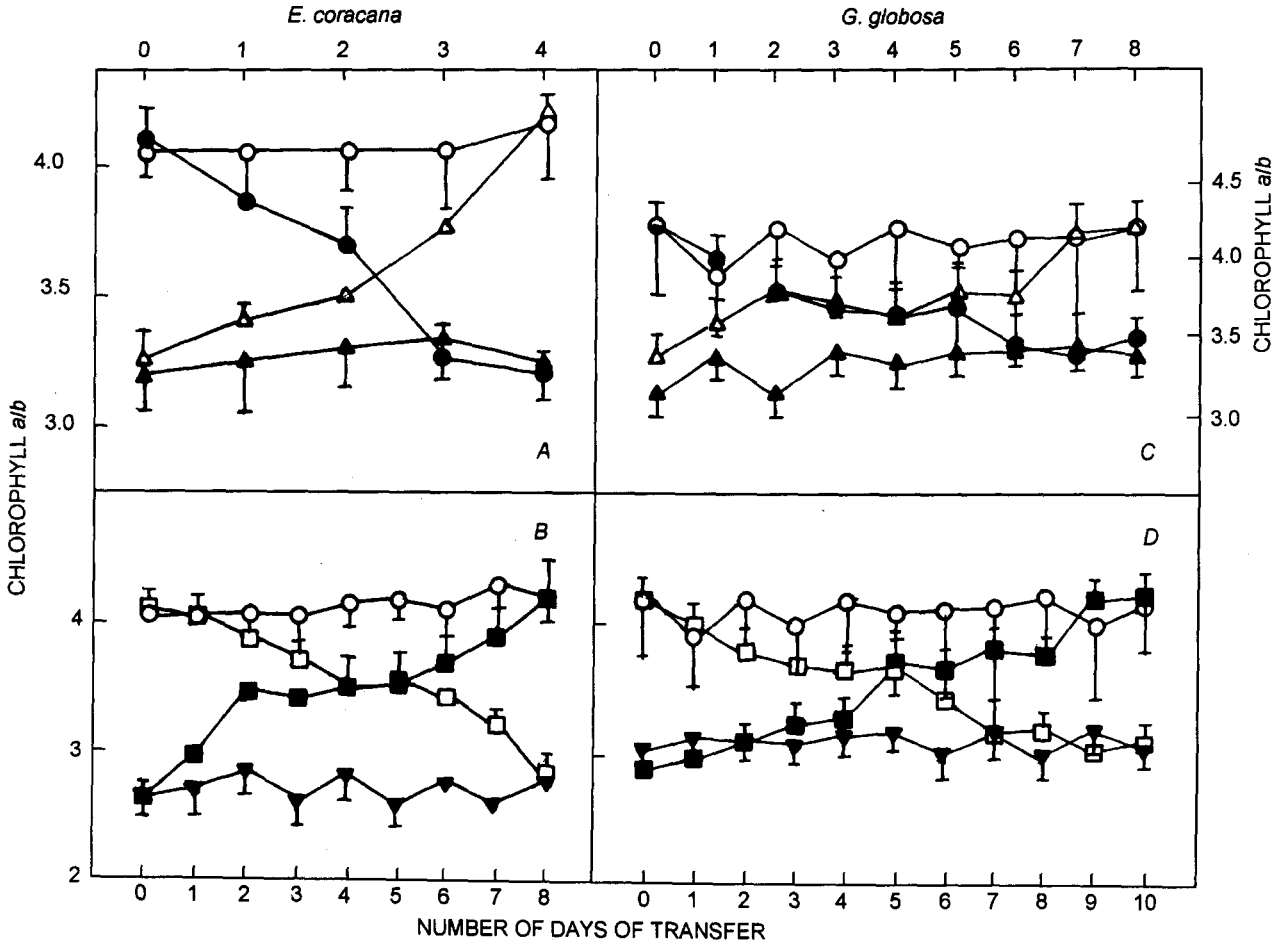

Fig. 1. Effects of limiting-irradiance on the chlorophyll *a/b* ratio in *E. coracana* (*A, B*) and *G. globosa* (*C, D*). Acclimation to reduced irradiance of 650 μmol m^{-2} s^{-1} (*A, C*) or 200 μmol m^{-2} s^{-1} (*B, D*) in HI (o), LI_1 (▲), LI_2 (▼), HI→LI_1 (●), LI_1→HI (Δ), LI_2→HI (■), and HI→LI_2 (□) plants.

The reduction in function of PS2 under reduced irradiances lead us to measure the electron transport rates under these conditions. The electron transport rates decreased in the LI_1 and LI_2 plants of both species in comparison with HI plants. A lesser change was observed in the whole electron transport chain comprising PS1 and PS2 in *G. globosa*, in response to reduction in growth irradiances, compared to that in *E. coracana* (Table 1). In HI→LI_1 and HI→LI_2 plants of *E. coracana*, the whole chain electron transport decreased by 57 and 72 %, respectively, while the decrease in similar *G. globosa* plants was 54 and 65 %, respectively. In LI_1→HI and LI_2→HI *E. coracana* plants, the electron transport increased by 130 and 256 %, respectively, whereas 118 and 184 % increase was observed in low irradiance grown *G. globosa* plants transferred to normal irradiance. PS1 electron transport rates decreased in HI→LI_1 and HI→LI_2 plants of *E. coracana* (63 and 77 %, respec-

tively) and *G. globosa* plants (52 and 73 %, respectively). In $LI_1 \rightarrow HI$ and $LI_2 \rightarrow HI$ plants of *E. coracana* and *G. globosa*, the PS1 electron transport rates increased. PS2 electron transport rates decreased to a greater extent compared to that of PS1 in $HI \rightarrow LI_1$ and $HI \rightarrow LI_2$ plants of both species. Likewise, the change in the PS2 rates was also higher in $LI_1 \rightarrow HI$ and $LI_2 \rightarrow HI$ plants compared to PS1 rates in both species.

The concentrations of Cyt *f* decreased in both species grown at reduced irradiances (Table 1). In $HI \rightarrow LI_1$ and $HI \rightarrow LI_2$ plants of *E. coracana*, the concentrations of Cyt *f* decreased by 41 and 78 %, respectively, while the decrease observed in similar *G. globosa* plants was 31 and 59 %, respectively. In $LI_1 \rightarrow HI$ and $LI_2 \rightarrow HI$ *E. coracana* plants the Cyt *f* concentrations increased by 69 and 361 %, respectively, whereas 46 and 148 % increases were observed in low irradiance grown *G. globosa* plants transferred to normal irradiance.

Table 1. Effect of reduced irradiance on the ratio of Chl fluorescence F_{690}/F_{735} at 77 K, the ratio Chl/cytochrome *f*, quantum efficiency of photosystem 2 (PS2) at room temperature and 77 K, photosynthetic electron transport rates [mol kg^{-1}(Chl) h^{-1}], cytochrome *f* concentrations [mol kg(Chl)$^{-1}$], and net photosynthetic rate [mol kg^{-1}(Chl) s^{-1}] in *E. coracana* and *G. globosa*. Values are means ± SD of ten independent measurements per trial and three such trials on the last day of acclimation to reduced irradiances. [a] = $p < 0.001$; [b] = $p < 0.005$.

	HI	LI_1	LI_2	$LI_1 \rightarrow HI$	$LI_2 \rightarrow HI$	$HI \rightarrow LL_1$	$HI \rightarrow LL_2$
				% decrease of control		% increase of control	
F_{690}/F_{735}							
E. coracana	0.40±0.02	0.85±0.04[b]	1.08±0.02[b]	52	67	113	165
G. globosa	0.51±0.09	0.87±0.04[a]	1.18±0.02[a]	41	54	70	121
Chl/Cyt *f*							
E. coracana	311±33	523±18[b]	1234±180[a]	40	77	68	346
G. globosa	326±34	485±58[b]	821±106[a]	32	60	48	103
				% increase of control		% decrease of control	
PS2 at room temperature							
E. coracana	0.76±0.06	0.36±0.06[b]	0.26±0.03[b]	111	192	52	62
G. globosa	0.81±0.09	0.52±0.04[a]	0.38±0.02[a]	55	113	36	53
PS2 at 77 K							
E. coracana	0.84±0.06	0.74±0.04[b]	0.63±0.06[a]	13	33	11	25
G. globosa	0.86±0.06	0.77±0.04[b]	0.69±0.04[a]	12	24	10	20
Whole chain ET							
E. coracana	960±136	417±56[b]	250±44[b]	130	256	57	72
G. globosa	942±148	432±48[a]	331±48[a]	118	184	54	65
PS1							
E. coracana	2827±434	1038±148[a]	637±120[a]	256	343	63	77
G. globosa	2142±178	1018±138[b]	556±105[a]	114	285	52	73
PS2							
E. coracana	184±19	53±12[a]	24±6[a]	247	660	71	86
G. globosa	142±20	58±8[a]	28±6[a]	144	407	59	80
Cyt *f*							
E. coracana	3.60±0.30	2.12±0.23[a]	0.78±0.08[a]	69	361	41	78
G. globosa	3.38±0.40	2.33±0.30[b]	1.36±0.16[a]	46	148	31	59
P_N							
E. coracana	44±6	22±3[b]	17±2[b]	108	177	51	72
G. globosa	21±3	16±2[a]	10±2[a]	40	87	33	42

The effect of reduced irradiance on P_N was measured to study the photosynthetic capacity under LI stress. P_N was lower in LI plants than in HI plants (Table 1). The rates decreased to a greater extent in $HI \rightarrow LI_1$ and $HI \rightarrow LI_2$ plants of *E. coracana* compared to that of similar *G. globosa* plants. The increase in P_N was also higher in $LI_1 \rightarrow HI$ and $LI_2 \rightarrow HI$ *E. coracana* plants compared to similar *G. globosa* plants. However, P_N was higher in control HI plants of *E. coracana* compared to that of *G. globosa*.

Discussion

The Chl *a/b* ratio decreased whereas the Chl fluorescence ratio at 77 K, F_{690}/F_{735}, and Chl/Cyt *f* ratios increased in both species under reduced irradiances (Fig. 1, Table 1) suggesting an increase in LHC2 (Lichtenthaler *et al.* 1982a,b, Terashima and Inoue 1984, Anderson 1986, Krause and Weis 1991). The Chl fluorescence ratio F_{690}/F_{735} at 77 K reflects changes in pigment-protein complexes (Šesták and Šiffel 1997) while the same ratio at room temperature is an indicator of the *in vivo* Chl content and is inversely correlated to the Chl content in a curvilinear manner (Lichtenthaler *et al.* 1990). The ratio at room temperature is frequently applied to determine the Chl content of leaves in a non-destructive way (Hák *et al.* 1990). The increase in LHC2 under reduced irradiances was greater for *E. coracana* compared to *G. globosa* suggesting necessity for the greater investment in Chl towards light-harvesting in former species compared to the latter one. The time taken for *E. coracana* plants to acclimate to reduced irradiances (650 and 200 μmol m^{-2} s^{-1}) was 4 and 8 d, respectively, whereas in *G. globosa* the time period was 8 and 10 d, respectively (Fig. 1). The difference in time period of acclimation to reduced irradiances between the two species is in contrast to our earlier observation that the time taken by carbon metabolism to acclimate to reduced irradiances (650 and 200 μmol m^{-2} s^{-1}) was identical (Sailaja and Rama Das 1995b). F_v/F_m was reduced in both plant species under reduced irradiances but the decline in the ratio was greater in *E. coracana* compared to that of *G. globosa* at both room temperature and at 77 K (Table 1). However, the fluorescence measurements at 77 K and field temperatures were not identical. This may be due to the lack of the effect of metabolic reactions on fluorescence at 77 K (Ögren and Öquist 1984, Sailaja and Rama Das 1996b). The F_v/F_m ratio denotes the excitation energy captured by the open reaction centres in the absence of photochemical quenching (Genty *et al.* 1989, Adams *et al.* 1990). Therefore, the decrease observed in F_v/F_m in the plants grown under reduced irradiances is an indication of a decrease in the excitation energy capture by the open reaction centres. The number of light-harvesting antennae increased (Fig. 1, Table 1) to optimise the function of open reaction centres offsetting the decrease in the excitation energy capture (Table 1). The assemblage of light-harvesting complex was apparently larger in *E. coracana* compared to *G. globosa* grown at reduced irradiances (Fig. 1, Table 1) to offset the extra decrease in the efficiency of excitation energy capture by the open reaction centres (Table 1). Thus, the present study agrees with our previous study on *Amaranthus* (Sailaja and Rama Das 1995a) that there is impairment in functional open reaction centres under sub-optimal irradiance because of lowered excitation energy capture due to more closed reaction centres. In contrast to that, an increase in the efficiency of energy transfer is observed in a shaded habitat (Björkman and Demmig 1987). However, the excitation energy capture did not change in *Silene dioica* grown at reduced irradiance (McKiernan and Baker 1991). The reduction in function of PS2 reaction centres under limiting-irradiance was restored to the full PS2 functional capacity when normal irradiance was provided along with a reduction in LHC2 (Table 1). Reduced antenna under high-irradiance in both species (Table 1) could lessen the susceptibility of damage to PS2 since plants with large light-harvesting antennae are exposed to greater photoinhibition of photosynthesis under high-irradiance (Anderson and Andersson 1988, Park *et al.* 1997).

The percent decrease in whole chain, PS1, and PS2 electron transport rates due to changes in growth irradiance varied in both species (Table 1). *E. coracana* showed greater reduction in electron transport rates compared to *G. globosa*. The electron transport in *E. coracana* was reduced more to match the extra decrease in the performance of photosystems and redox carriers and was restored to normal when normal irradiance was available (Table 1). The dynamic modulation of electron transport in response to reduced growth-irradiance in *E. coracana* and *G. globosa* reiterates that species specificity exists to fine tune the process of photo-synthesis in plants acclimated to reduced irradiance. Under limiting irradiance the impairment of PS2 electron transport was higher compared to that of PS1 (Table 1) confirming the fact that PS2 is more susceptible to stressing irradiance over PS1 (Anderson and Andersson 1988, De la Torre and Burkey 1990, Sailaja and Rama Das 1996a). The observed smaller change in function of the Cyt b_6/f complex in *G. globosa* compared to that of *E. coracana* might be related to less decline in the electron transport and the functional PS2 reaction centres under reduced irradiances (Table 1). This in turn accounts for the observed more efficient P_N in *G. globosa* compared to that of *E. coracana* under limiting irradiances (Table 1). However, control HI plants of *E. coracana* had higher P_N consistent with the higher amounts of Cyt b_6/f compared to HI plants of *G. globosa* (Table 1). The more efficient photosynthetic performance of *G. globosa* was likely to be due to the increased allocation of metabolic energy towards assembly of the Cyt b_6/f under limiting irradiance, unlike *E. coracana* which shows a greater investment in the light-harvesting complexes. The prominent regulatory role of Cyt *f* in LI plants in determining P_N is in agreement with previous studies (Anderson 1992, Price *et al.* 1995, Evans 1997).

We identified two modes of acclimation to reduced irradiances: (*1*) the *G. globosa* type which combines modulation of composition and function of thylakoid membrane to relatively higher performance, and (*2*) the *E. coracana* type in which a greater investment in the light-harvesting apparatus is employed in order to achieve

a reasonable acclimation level. *Amaranthus*, an NAD-ME C_4 dicotyledon plant grown under the same irradiances (650 and 200 μmol m^{-2} s^{-1}) showed acclimation similar to *E. coracana* type which is an NAD-ME monocotyledon species (Sailaja 1994, Sailaja and Rama Das 1995a, 1996a) indicating that the observed difference between the two species in the present study is not because of structural differences between monocotyledon and dicotyledon plants. NADP-ME C_4 plants have superior mode of acclimation compared to NAD-ME type C_4 plants. The observation is meaningful in view of the fact that NADP-ME is considered to be an advanced form of C_4 that evolved from the relatively primitive NAD-ME type. Carbon assimilation and photosynthetic acclimation are co-ordinated under reduced growth irradiance (Walker and Sivak 1986). End product of carbon assimilation is crucial in biochemical feed back regulation of carbon assimilation by depriving the orthophosphate concentrations in chloroplasts of low irradiance grown plants (Walker and Sivak 1986). The orthophosphate is essential for regulating the electron transport and carbon metabolism (Walker and Osmond 1986, Walker and Sivak 1986, Foyer 1988). Efficient recycling of the orthophosphate is not possible under reduced ATP conditions (Walker and Sivak 1986). PS1 electron transport rates decreased under the limiting irradiance in both *E. coracana* and *G. globosa* (Table 1) resulting in reduced ATP content and probable inefficient recycling of orthophosphate. The greater efficiency of orthophosphate recycling in NADP-ME C_4 plants compared to the NAD-ME C_4 plants due to a lesser reduction in the PS1 electron transport rates (Table 1) could account for the observed more efficient photosynthetic performance at reduced growth irradiances. The efficiency of orthophosphate recycling in different types of C_4 plants under limiting-irradiance needs to be investigated further.

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