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Irradiance influences contents of photosynthetic pigments and proteins in tropical grasses and legumes

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Abstract

Three tropical range grasses (*Cenchrus ciliaris*, *Dichanthium annulatum*, and *Panicum antidotale*) and two range legumes [*Macroptilium atropurpureum* (siratro) and *Stylosanthes hamata* (stylo)] were grown under four irradiances, *i.e.* 100 (I_{100}, control), 75 (I_{75}), 50 (I_{50}), and 25 (I_{25}) % of full sunlight. Accumulation of chlorophyll (Chl) *b* increased but that of Chl *a* decreased under low irradiances. The greater accumulation of Chl (*a*+*b*) in grasses (particularly in *D. annulatum* and *P. antidotale*) under shade predicted their shade adaptability. Among legumes *Stylosanthes* was more adaptive to the shade than *Macroptilium* due to its higher accumulation of Chl (*a*+*b*). Significant difference in the accumulation of carotenoids under I_{25} over I_{100} was observed in all the species, which shows the increase in quality of the fodder under limited irradiance. There was a significant decrease in soluble protein content in *C. ciliaris* under I_{75}, however, no significant difference in protein content was observed under I_{50} and I_{25}, which was also reflected in the SDS pattern with the reduction in content of polypeptides at I_{75} and following increase at I_{50} and I_{25}. This was possibly due to reduction of light-induced protein at I_{75} and then expression of the stress-induced protein at further reduction of irradiance. Peroxidase activity in *C. ciliaris* increased with the decrease in irradiance and its isozyme pattern showed differences among all treatments, which indicated the role of different peroxidase isoforms at different irradiances.

Additional key words: carotenoids; *Cenchrus*; chlorophyll; *Dichantium*; *Macroptilium*; *Panicum*; polyacrylamide gel electrophoresis; *Stylosanthes*.

Introduction

Morphological development of plants, leaves, and chloroplasts largely depends on the irradiance under which the plants are grown (Boardman *et al.* 1975, Lichtenthaler 1979, 1981). This development greatly depends on the amount of photons available during growth. Under high growth irradiance plants react with a strong growth response and under low growth irradiance with a weak growth response (Lichtenthaler 1981). The shade-type chloroplasts of shade leaves, low-irradiance leaves, and shade plants are characterized by much larger grana stacks and higher stacking degree (Anderson *et al.* 1973, Guillot-Salomon *et al*. 1978, Lichtenthaler *et al.* 1981) than the sun-type chloroplasts, which have much less chloroplast lamellae. High-irradiance chloroplasts are represented by higher ratio of chlorophyll (Chl) *a/b* and lower ratios of xanthophylls/carotene (x/c) and Chl *a*/prenylquinones (Lichtenthaler 1979, Lichtenthaler *et al*. 1981).

The characteristics of photosynthetic reactions differ between shade tolerant species grown in shade and shade-intolerant species acclimated to higher irradiance (Björkman 1981): acclimation responses occur among genetically uniform plants grown at different irradiances and among leaves of individual plants acclimated to different irradiance. Bond *et al.* (1999) determined the acclimation of foliage physiology along the canopy irradiance gradient in conifers of varying shade tolerance. Photon-saturated net photosynthetic rate (P_{Nmax}) and Chl *a/b* ratios were higher in foliage of canopy positions exposed to higher irradiance as compared to shaded crown layers. The shade tolerant species showed relative shade-type characteristics at a given radiation environment, both P_{Nmax} and Chl *a/b* ratio were lower in needles of the shade tolerant species. In higher plants, the amount of incident solar radiation available during growth produces distinct differences in the composition, function, and structure of chloroplasts (Leong and Anderson 1984, Lechowicz *et al*. 1986).

We tried to define typical differences in the accumulation of photosynthetic pigments, SDS protein profiles, and isozymes between sun-type and shade-type leaves of some tropical grass and legume species.

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Materials and methods

Plants: The experiment was conducted at the Indian Grassland and Fodder Research Institute, Jhansi, India (25°27'N, 78°35'E, 271 m a.s.l.). The soil was clay loam in texture, neutral in reaction (pH 6.54), and non saline (EC 0.29 ds m^{-1}). The contents of organic carbon (0.48 %) and available nitrogen (23.52 g m^{-2}) in the surface soil were low. The available phosphorus and potassium contents were in the medium range [1.27 g(P) m^{-2} and 27.88 g(K_2O) m^{-2}]. The species studied were tropical range grasses *Cenchrus ciliaris, Dichanthium annulatum,* and *Panicum antidotale*, and tropical legumes *Macroptilium atropurpureum* and *Stylosanthes hamata*. These species are in wide use in tropical areas and form a successful mixed pasture.

Grass establishment: Seedlings of grass species were grown in the seedbeds. One-month-old seedlings were transplanted in the field with 2 m^2 plots and 50×50 cm distance from plant to plant and row to row. All plots were in full sunlight for one season for proper establishment of tussocks. After one season low irradiance treatment was given. The legume seeds were sown directly to the experimental plots of the same size as grasses before the onset of monsoon**.**

Four irradiances, *i.e.* 100 (I_{100}, control), 75 (I_{75}), 50 (I_{50}), and 25 (I_{25}) % of full sunlight were applied for each subplot, each with three replications. Solar irradiance in I_{100} was 1 200–1 400 μmols m^{-2} s^{-1} throughout the growing season. The agro shade nets were mounted on wooden frames 10×5 m set 2 m above the ground. At the eastern and western ends of each screen the shade was extended for extra 1 m and angled at 45° to exclude most of the early morning and late evening sun from plots. The shading treatment was imposed before the onset of monsoon (June–July) as the growing season of these tropical grasses and legumes ranges from the onset of monsoon (June–July) to the beginning of the winter (November–December).

Photosynthetic pigments: Chl *a* and *b* contents were determined by extraction in dimethyl sulphoxide (DMSO) using a non-maceration technique of Hiscox and Israelstam (1979). Carotenoids (Cars) were extracted following the method of Jensen (1978).

Enzyme activity and soluble proteins: Fresh leaves were ground in a pre-chilled pestle and mortar with 1 : 2 (m/v) 50 mM phosphate buffers (Na_2HPO_4/NaH_2PO_4, pH 7.0). Homogenate was centrifuged at 4 °C for 20 min at 15 000×*g*. This extract was used for estimating soluble protein following the procedure of Lowry *et al.* (1951). Peroxidase activity was estimated in the same supernatant by an increase in absorbance at 436 nm per s in a mixture of 3 cm^3 phosphate buffer (pH 7.0), 1 cm^3 guiacol solution (2.4 mg per cm^3), 0.1 cm^3 enzyme extract, and 30 mm^3 hydrogen peroxide (Chance and Machly 1955). The activity was expressed in unit per mg protein, whereas one enzyme unit was defined as a change of 0.1 absorbance per min caused by the respective enzyme aliquot.

SDS-polyacrylamide gel electrophoresis (PAGE) was performed using 12 % gel according to Laemmli (1970) with modification. An aliquot (15 cm^3) of the above extract was mixed with 2X sample buffer (0.25 M Tris-Cl, pH 6.8; 0.2 % sodium dodecyl sulphate, SDS, 10 % glycerol, 10 % β-mercaptoethanol, and 0.002 % bromophenol blue) and electrophoresed at 30 mA on a 1.0×1.5 mm gel.

Isozymes: Fresh leaf sample was extracted with 1 : 2 (m/v) volume of Tris-Cl buffer (pH 7.6) containing 5 mM β-mercaptoethanol. For peroxidase and esterase isozymes, anionic PAGE was used (Davis 1964) by loading approximately 150 μg protein per sample. For staining peroxidase isozymes, the gel was incubated at room temperature with 0.2 M acetate buffer (pH 5.6) containing 0.001 % benzidine and 5 % H_2O_2 solution was added slowly with gentle shaking. Esterase isozymes were stained with 50 mM phosphate buffer (pH 6.0) containing 0.02 % α-naphthyl acetate (dissolved in 2 cm^3 of 60 % acetone) and 0.05 % Fast Blue RR salt.

Results

Photosynthetic pigments: The photosynthetic pigment contents were estimated in the leaves before harvest of the crop at 50 % flowering stage. Chl (*a*+*b*) content was higher in the plants under shade than in the sun (Fig. 1*A*). Average Chl *a* content in dry mass was higher in I_{100} or I_{75} than in I_{50} and I_{25} plants. There was a considerable species variation in Chl *a*/*b*, with a tendency for reduction under shade (Table 1). *P. antidotale* did not show the same trend.

Cars: The accumulation of Cars showed an increasing trend when the irradiance decreased from 100 to 25 %. The difference was significant comparing I_{25} and I_{100} *D. annulatum* and *P. antedotale*, however, no significant difference was found comparing I_{75} and I_{50} plants of *C. ciliaris* and *S. hamata*. *M. atropurpureum* did not show a difference at I_{50} and I_{25} (Fig. 1*B*). The accumulation of Cars showed an increasing trend with a decrease in irradiance. *D. annulatum* accumulated highest amount of Cars followed by *P. antidotale* and *C. ciliaris*. Among the legumes, the highest amount of Cars was accumulated in *S. hamata* followed by *M. atropurpureum*.

Table 1. Chlorophyll *a/b* ratio as influenced by different irradiances (I_{100} to I_{25}).

		I_{100}	I_{75}	I_{50}	I_{25}
Range grasses	*Cenchrus ciliaris*	6.55	5.44	5.97	4.99
	Dichanthium annulatum	5.64	4.58	4.97	4.52
	Panicum antidotale	4.67	6.74	3.09	5.26
	Mean	5.62	5.59	4.67	4.92
Range legumes	*Macroptelium atropurpureum*	4.23	4.04	3.71	3.87
	Stylosanthes hamata	4.75	4.33	4.30	4.19
	Mean	4.49	4.19	4.01	4.03
	CD at p=0.05	Irradiance, *I*: 0.435, plant species, *S*: 0.622, *S*×*I*: 0.853			

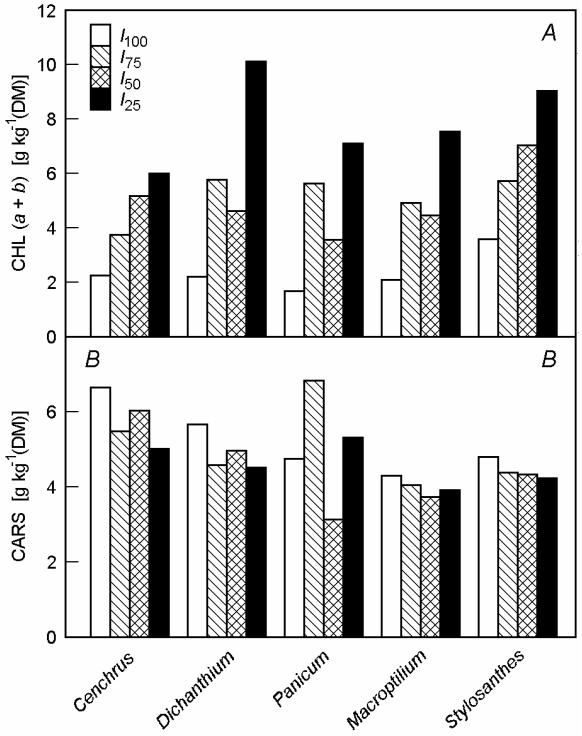

Fig. 1. Contents of (*A*) chlorophyll, Chl (*a*+*b*) and (*B*) carotenoids, Cars in the leaves of grasses and legumes under different irradiances. Means of three replications. (*A*): L.S.D. p<0.05, irradiance (*I*) = 0.164, plant species, *S* = 0.129, *S*×*I* = 0.204. (B): L.S.D. p<0.05, *I* = 0.070, *S* = 0.059, *S*×*I* = 0.092.

Protein profile and isozymes: Soluble protein content in *C. ciliaris* leaves decreased marginally at I_{75} but increased at I_{50} and I_{25} (Table 2). This was reflected in the SDS-PAGE pattern with the reduction of polypeptides at I_{75} and then increase at I_{50} and I_{25} (Fig. 4*A*). This was possibly due to reduction in content of light-induced proteins at I_{75} and then expression of the stress-induced protein at further reduction of irradiance. Peroxidase is a stress-induced enzyme, which helps in combating the stress. Peroxidase activity increased with decrease in irradiance (Table 2) and its isozyme pattern showed differences among all the treatments (Fig. 4*B*). This indicates the role of different peroxidase isoforms at different irradiances. Esterase pattern showed the existence of two low irradiance sensitive isoforms (Fig. 4*C*).

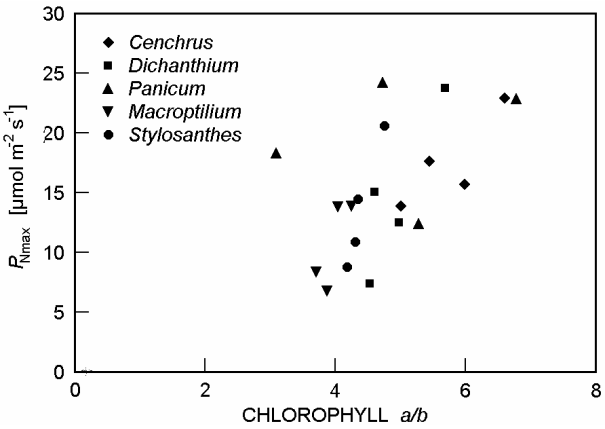

Fig. 2. Differences in chlorophyll *a/b* ratio plotted against the differences in maximal net photosynthetic rate, P_{Nmax}.

Discussion

Photosynthetic pigments: Chl (*a*+*b*) content was higher in plants grown under shade than in sun (Fig. 1*A*). The increased pigment content in shaded leaves is attributed to the increase in number and size of chloroplasts, the amount of Chl per chloroplast, and better grana development (Boardman 1977). Under the shade, the accumulation of Chl *b* was higher than in plants grown under I_{100}. Shaded plants have a higher relative content of Chl *b* than Chl *a* (Singh 1994). However, under different irradiances Chl *a* behaved variably. *P. antidotale* accumulated more Chl *b* than other grasses and legumes, indicating its potential for shade adaptation.

Lichtenthaler (1981) found in radish seedlings a significantly higher Chl *a/b* in plants grown under high (HI) that low (LI) irradiance, but Chl content per dry mass and the height and width of grana stacks were in turn always greater in the LI-leaf. Higher Chl *a/b* in HI-leaves indicates lower amount of light-harvesting Chl-protein, which was shown for HI-leaves of *Raphanus* and barley (Armond and Arntzen 1977, Burke *et al.* 1979). Chl *a/b*

ratios are typically higher in sun leaves than shade leaves (Boardman 1977**,** Anderson *et al.* 1988): (*a*) sun leaves possess higher capacities for photosynthesis and (*b*) place less emphasis on light-harvesting. Chl *a/b* can rise as a result of different ratios of photosystem (PS) 2 cores to peripheral light-harvesting complexes (Anderson and Osmond 1987). Nevertheless, higher Chl *a/b* ratios in sun than shade are sometimes found, *e.g*. in *Juglans regia* (Atanasova *et al*. 2003). This means a lesser emphasis in photon collection *versus* PS2 photochemistry. In addition, Chl *a/b* ratios are higher in Chl complexes of PS1 compared to those of PS2 (Thayer and Björkman 1992). Thus the higher Chl *a/b* ratios in the open condition indicate differences in either the ratio of PS2 core to light-harvesting complexes or the ratio of Chl complexes associated with PS1 *versus* PS2 or both. Adjustments of the leaf pigment composition in response to irradiance have been described in numerous studies. Chl-based contents of photo-protective Cars and Chl *a/b* ratios are higher in sun *versus* shade leaves of individual plants (Thayer and Björkman 1990, García-Plazaola and Becerril 2000, Lichtenthaler *et al*. 2000, Hansen *et al*. 2002) and in sun-grown *versus* shade-grown plants (Logan *et al.* 1998). Chl *b* is enriched in outer antennae, relative to the core complexes (CC) of PS1 and PS2 and in PS2 *versus* PS1. A higher contribution of outer antennae Chl to the total Chl, leading to lower Chl *a/b* ratios, enhances the efficiency of photon capture under limited photon supply.

Corresponding results were obtained by Hansen *et al.* (2002) on deciduous tree species, *Fagus sylvatica* and Q*uercus petrea*, in a mixed beech/oak forest: shade-type pigment characteristics for shade leaves relative to sun leaves. At a given irradiance, leaves of the more shade-tolerant beech showed lower Chl *a/b* ratios than those of the less shade tolerant oak.

The average Chl amount per leaf fresh mass was higher under shade compared to full sunlight, suggesting a higher investment in pigment-protein complexes. The variability in Chl content among the species was much profound and possibly arising from content of water and amount of non-photosynthesising tissues. There was substantial variation between species in the extent and nature of alteration in photosynthetic characteristics. This is demonstrated in Fig. 2 (P_{Nmax} *vs*. Chl *a/b*): these parameters are commonly used when monitoring the sun/shade photosynthetic characteristics. The changes in P_{Nmax} under shade are often associated with altered ribulose-1,5-bisphosphate carboxylase/oxygenase content under saturating CO_2 concentration (Stitt 1986, Seemann *et al.* 1987).

Relationship between Cars (*x*+*c*) composition and Chl (*a*+*b*): The amount of Cars increased with increasing Chl

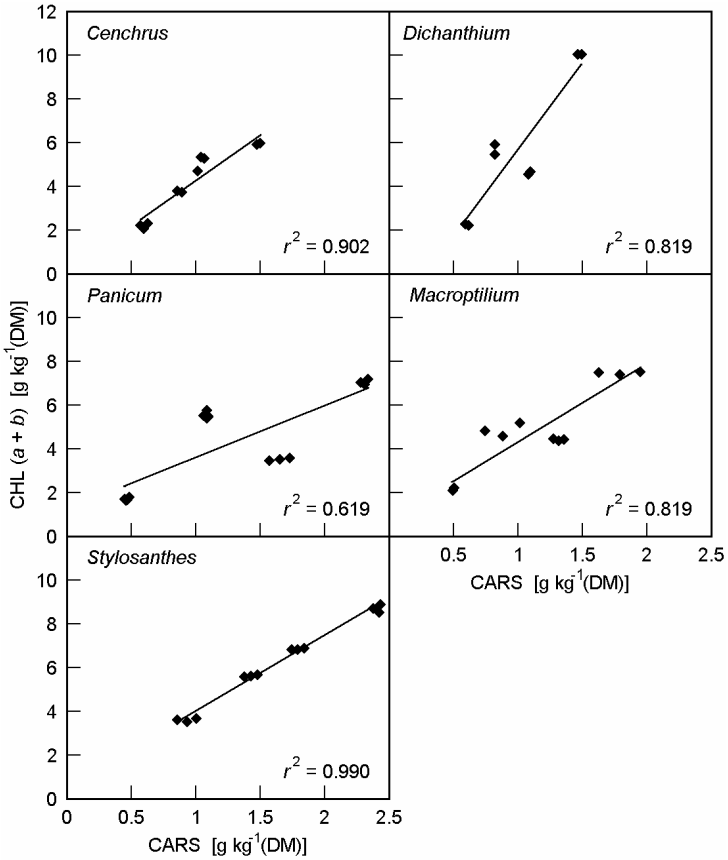

Fig. 3. Relationship between contents of carotenoids, Cars and total chlorophyll, Chl (*a*+*b*) in different forage species.

(*a*+*b*) (Fig. 3): these trends were very similar to those with decreasing % of absorbed photons that could be utilised in PS2 photochemistry. The relationship between Cars and Chl (*a*+*b*) also was similar as that between Cars and % of absorbed photons that could be utilised in PS2 phytochemistry. The different antenna complexes of PS2 contain different amounts of Cars (violaxanthin, antheraxanthin, and zeaxanthin) (Bassi *et al.* 1993)**.** The minor, proximal light-harvesting Chl proteins (CPs) CP24, CP26, and CP29 (Peter and Thornber 1991) are enriched in xanthophyll cycle components compared to the major, peripheral light-harvesting complex LHC2 (Bassi *et al.* 1993, Falbel et al. 1994, Lee and Thornber 1995). Our results are more consistent with the assumption that these

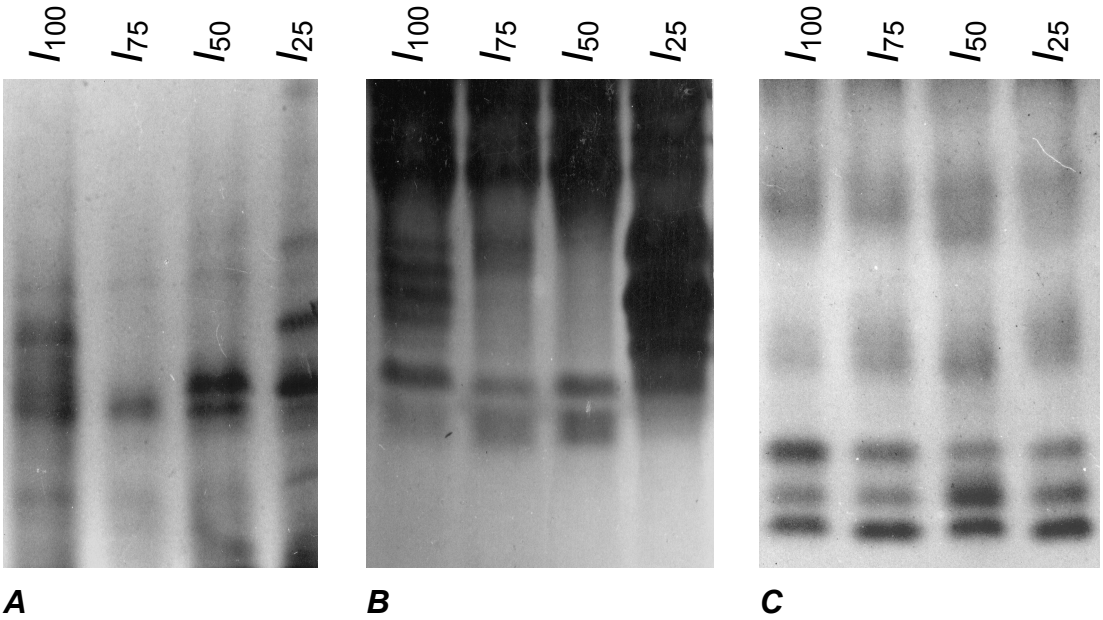

Fig. 4. Patterns of (*A*) SDS proteins, (*B*) esterase isozymes, and (*C*) peroxidase isozymes in *Cenchrus ciliaris* under different irradiances, I_{100} (full) to I_{25} (25 % of full).

Table 2. Soluble protein content [g kg^{-1}(FM)] and peroxidase activity [U mg^{-1}(protein)] in the leaf extract of *C. ciliaris* at different irradiances (I_{100} to I_{25}).

Irradiance	Soluble protein	Peroxidase activity
I_{100}	80.64	36.70
I_{75}	58.59	41.25
I_{50}	74.13	49.45
I_{25}	79.10	56.52
CD at 5 %	18.67	2.59

species predominantly adjust the composition of complexes within PS2 (or possibly PS1) in response to changes in the irradiance stress. Higher Chl (*a*+*b*) and more Cars with increasing irradiance are consistent with a lesser emphasis on the major, peripheral light-harvesting complexes and a maintenance of the inner CPs enriched in xanthophyll cycle components.

Protein profile and isozymes: Soluble protein content decreased marginally at I_{75} but increased at I_{50} and I_{25} (Table 2). This was reflected in the SDS-PAGE pattern with the reduction of polypeptide at I_{75} and then increase at I_{50} and I_{25} (Fig. 4*A*). This is possibly due to reduction of light-induced protein at I_{75} and then expression of the stress induced protein at further reduction of irradiance. Native protein profile studies showed that under low irradiance there was a reduction in the contents of some proteins (Viji *et al.* 1997). They observed the induction of light-harvesting Chl-protein complex in shade tolerant cultivar of rice. But irradiance was not mentioned in the above studies. Crude protein content was higher at shade in *Codonopsis lanceolata* (55 % shade; Lee *et al*. 1996), different grasses (Mika *et al*. 1998, Park *et al.* 1998), and oat seeds (Nandal and Bisla 1995). Palis and Bustrillos (1976) recorded the increase in protein content and decrease in saccharide content in the sorghum seed grown under I_{25} and I_{50}. Vyas *et al*. (1996) reported that 25 as well as 50 % shading increased the plant water status, transpiration rate, and Chl, protein, and amino acid contents in cluster bean (*Cyamopsis tetragonoloba*). Some enzyme activities (nitrate reductase and glutamate dehydrogenase) increased at 25 % shade but glutamate dehydrogenase activity decreased at 50 % shade. Protein contents were decreased at 30, 50, or 70 % shading in *Aster scaber* and *Ligularia fischeri* (Hong *et al*. 1996). However, the above papers show that the contents of protein

and enzymes under shade depend upon plant species and hence, a generalization is not possible.

Peroxidase activity increased with decrease in irradiance and its isozyme pattern was different among all the treatments (Fig. 4*C*). This indicates the role of different peroxidase isoforms at different irradiances. Isoenzyme analysis of peroxidase showed the induction of a new band in rice cultivar IR 20 subjected to low irradiance (Viji *et al*. 1997). In our study, the increases in protein content and in peroxidase activity were adaptations to shade. The higher accumulation of Chl *b* in *C. ciliaris* under shade (Baig *et al*. 1998) also predicted its shade adaptability. Esterase pattern showed the existence of two low irradiance sensitive isoforms (Fig. 4*B*). Specific esterase band under higher stress (low irradiance) must have a specific role in shade tolerance and thus can be used as a marker for selecting the stress tolerant grasses.

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