Modulation of thylakoid membrane composition and function during acclimation to limiting growth light levels in *Amaranthus hypochondriacus* L. an NAD-ME C_4 plant

Thesis Submitted for the Degree of
DOCTOR OF PHILOSOPHY
UNIVERSITY OF HYDERABAD

BY
M.V. SAILAJA
(Enrolment No: JA 1914)

DEPARTMENT OF PLANT SCIENCES
SCHOOL OF LIFE SCIENCES
UNIVERSITY OF HYDERABAD
HYDERABAD - 500 046, INDIA

March 1994

D E C L A R A T I O N

The candidate declares that this work has been carried out by her under the supervision of Prof. V.S. Rama Das, Professor, School of Life Sciences, University of Hyderabad, Hyderabad - 500 134, India, and that this work has not been submitted for any degree or diploma of any other University or Institution.

M.V. Sailaja

Ms. M.V. Sailaja
Candidate

Prof. V.S. Rama Das
Supervisor.

UNIVERSITY OF HYDERABAD

Phones : 253901 Extn : 4506
FAX : 91-040-253145
Telex : 04292-205 UHYD IN

C E R T I F I C A T E

This is to certify that the thesis entitled "Modulation of thylakoid membrane composition and function during acclimation to limiting growth light levels in *Amaranthus hypochondriacus* **L. an NAD-ME C_4 plant"** is based on the results of the work done by Ms. M.V. Sailaja for the degree of Doctor of Philosophy under my supervision. This work has, not been submitted for any degree or diploma of any other University or Institution.

Prof. V.S. Rama Das *FKA.FASC.*
Supervisor
School of Life Sciences

Prof. A.R. Reddy
Head
Department of Plant Sciences
School of Life Sciences

Prof. ARJULA R. REDDY
Head Dept. of Plant Sciences
School of Life Sciences
University of Hyderabad.
HYDERABAD-500 134 India

Prof. N.C. Subrahmanyam *FNA.FNAAS*
Dean
School of Life Science

A C K N O W L E D G E M E N T S

It gives me immense pleasure to express my deep sense of gratitude to my mentor Prof. V.S. Rama Das for his guidance, invaluable suggestions and stimulating discussions during the study period. He is not only an academician of great repute, but a generous person who is sympathetic and understanding towards the problems faced during the tenure of my study.

1 am thankful to Prof. N.C. Subrahmanyam. Dean. School of Life Sciences for providing the necessary infrastructure for carrying out my research work. I am indebted to him for enabling me to use his laboratory facilities for carrying out Northern-blot experiment.

I am thankful to Prof. A.R. Reddy. Head. Department of Plant Sciences for providing required facilities to carryout my work. I am extremely thankful to Dr. M.N.V. Prasad for his kind help and cooperation during the course of my research work.

My sincere thanks to Dr. K.V. Reddy. Professor-in-charge of C.I.L. for extending the EPR-spectrometer facility. 1 am thankful to Mr. Manjunath and Mrs. M. Nirmalananda lor their help in EPR spectroscopy.

I am extremely thankful to Prof. M. Sigiura. Japan. for generously providing the psbA gene clone.

I am thankful to my friends Dr. Mrs. Shubhra Gupta. Dr. Ms. G. Jyothi. Ms. N. Karunasree. Ms. P. Padma and Ms. A.S. Sailaja for their valuable help during the course of my work. 1 am thankful to Ms. S. Rama Devi. Mr. T. Ham Kumar. Mr. CH. Nagesh. Mr. P. Kranthi Kumar and Mr. George Thomas for their timely help.

I thank Mr. K. Srinivas for typing this manuscript.

Financial assistance from U.G.C. (India) is gratefully acknowledged.

1 thank my parents, uncle, aunt and my brothers for their immense support during the course of my work.

LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate.
BSA	Bovine serum albumin.
BQ	Benzo quinone.
DCMU	3-(3,4-Dichlorophenyl) 1,1-Dimethyl Urea.
DCP1P	2,6-Dichlorophenolindophenol.
DEAE	Diethyl aminoethyl.
DNA	Deoxyribonucleic acid.
DTT	Dithiothreitol.
EDTA	Ethylenediamine tetra acetic acid.
F_o	Ground level fluorescence.
F_m	Fluorescence maximum.
F_v	Variable fluorescence.
HEPES	4-(-2-(Hydroxyethyl) Piperazine-1-ethane sulfonic acid).
kDa	Kilodalton
LHCII	Light harvesting complex of PSII
LHCPI	Light harvesting complex of PSI.
MES	2-Morpholinoethane sulfonic acid.
MV	Methyl viologen.
MOPS	Morpholino propanesulfonic acid.
NAD	Nicotinamide adenine dinucleotide.
NAD-ME	NAD-Malic enzyme.
NADU	Nicotinamide adenine dinucleotide (reduced).
NADP	Nicotinamide adenine dinucleotide phosphate.

NADPH	Nicotinamide adenine dinucleotide phosphate (reduced).
NADP-ME	NADP-Malic enzyme.
PEP	Phosphoenol pyruvate.
RNA	Ribonucleic acid.
RUBISCO	Ribulose bisphosphate Carboxylase oxygenase.
SDS	Sodium dodecyl sulphate.
SiMo	Silicomolybdate.
Tris	Tris (hydroxy methyl) aminomethane.
TRICINE	N-Trix-(Hydroxymethyl) methyl glycine.

C O N T E N T S

INTRODUCTION

Photosynthesis is a fundamental process of life on the earth involving the conversion of radiant energy of sun into chemical energy. Green plants are primary harvesters of solar energy and therefore are biological sun traps.

Plants growing in natural environment are often unable to express their full genetic potential for growth and reproduction due to either unfavourable soil conditions or climatic variations including drought, water logging and suboptimal levels of temperature and light intensity (Boyer 1982). An understanding of the mechanisms by which plants cope with adverse environments is of great importance in the context of photosynthesis and plant productivity. Therefore, investigations of photosynthetic acclimation are highly relevant not, only to gain basic knowledge but also for possible crop yield improvement. For example the mechanisms for drought resistance were used for improving yield in soybean (Meyer and Boyer 1972; Morgan 1977). The photosynthetic adaptive strategies for stressful environmental conditions in nature enabled the plants in death valley to survive (Björkman and Badger 1978). Accordingly it is expected that studies of photosynthetic adaptations under field conditions would produce useful results applicable to improvement programmes in crop yield (Das and Reddy 1988).

Under stressful environmental conditions plants have developed alterations at the molecular level (acclimation or adaptation) since they are sedentary and cannot escape unfavourable environmental conditions (Anderson and Andersson 1988). Growth light level is a crucial determining factor for the photosynthetic performance. It is known that under natural conditions limiting light intensities are frequently encountered due to daily fluctuations in solar irradiance, seasonal changes and mutual shading of leaves.

Adaptation or acclimation to light limiting conditions involve several changes in the photosynthetic apparatus. Adaptations to irradiance levels involve alterations in the genome (Ramus 1981) whereas acclimation is regarded as the expression of the adjustment that an organism can make to its environment within the limits of genome (Levy and Gnatt 1988; Falkowski and Laroche 1991).

Elucidation of the molecular organization of photosynthetic membranes (thylakoids) is needed to understand their modifications during adaptation/acclimation to reduced irradiances. Thylakoids are arranged in a continuous network of single nonappressed membrane interconnected with closely connected appressed membranes called the grana stacks. Only the outer surface of the nonappressed membrane is in direct contact with Chloroplast stroma where carbondioxide fixation occurs. Electron transport from water to $NADP^+$ requires participation of discrete multiprotein membrane complexes *viz.* 1) Photosystem II complex (PSII). 2) Cytochrome b_6/f complex. 3) Photosystem I complex (PSI). 4) ATP synthetase complex. These complexes are linked with each other through laterally mobile plastoquinone (PQ) pool and plastocyanin (PC). The protons from water and plastoquinone pool deposited in the lumen generate electrochemical energy for ATP synthesis by ATP synthetase in CF_o particles. Specific light harvesting pigment protein complexes LHC II_b, LHC II_a, LHC II_c and LHC II_e (Peter and Thornber 1991) are associated with Photosystem II and LHC I with PSI (Wynn *et al.* 1989) respectively. Both ATP synthetase and PSI complex are located in nonappressed regions of thylakoid membrane. Cytochrome b_6/f complex is distributed in both regions (Goodchild *et al.* 1985a). Most of Photosystem **II** and LHC **II** are located in appressed regions of thylakoids (Goodchild *et al* 1985b). Hence there is spatial segregation of most of **PSI** complex from **PSII** complex. The modulation of components of thylakoids membranes and their

function under reduced irradiances is of vital importance.

Plants have developed long and short term strategies of acclamation (Anderson and Aridersson 1988). The long term acclimation strategy requires changes in the composition of the photosynthetic membrane, while the short term acclimation involves reorganization of existing membrane components.

Long term acclimation to reduced irradiance

Pigment composition and content changes in thylakoid membranes grown under different irradiances is well documented (Björkman 1973; Boardman 1977; Björkman 1981; Lichtenthaler 1981; Melis and Harvey 1981; Leong and Anderson 1984a; Lichtenthaler and Meier 1984; Tobin and Silverthorne 1985; Davies *et al.* 1986; Chow and Anderson 1987a; Evans 1987a). Obligate shade plants have more chlorophyll per Chloroplast (Anderson *et al.* 1973) and lower chlorophyll *a/b* ratio compared to sun plants (Björkman 1973; Boardman 1977; Lichtenthaler 1981). Plants grown under high irradiance have high chlorophyll *a/b* ratios compared to plants grown under similar reduced irradiance levels (Lichtenthaler *et al.* 1982; Leong and Anderson 1984a; Chow and Anderson 1987a; Evans 1987a). Acclimation to reduced irradiance is seen in leaves of the lower strata of canopy (Lichtenthaler 1981; Burkey and Wells 1991; Evans 1993a). The chlorophyll a/b ratio of the bottom canopy decreases compared to leaves in the top most canopy. In a bifacial leaf, chlorophyll *a/b* ratio decreased on the lower surface compared to upper surface of the leaf (Terashima and Inoue 1984). The composition and content of carotenoids change during acclimation to reduced irradiances. High proportion of β carotene, reduced xanthophylls especially lutein and neoxanthin have been reported from plants grown under reduced irradiance levels (Lichtenthaler *et al.* 1982; Thayer and Björkman

1990). β carotene is mainly associated with core chlorophyll protein complexes while lutein and neoxanthin are associated with light harvesting chlorophyll protein complexes (Lichtenthaler 1981; Braumann *et al.* 1982; Eskins *et al.* 1985). The above fluctuations in xanthophyll/β carotene and chlorophyll *a/b* ratios could indirectly refer to alteration in core proteins and light harvesting pigment protein complexes during acclimation to reduced irradiances. Chlorophyll *a/b* ratio and Fluorescence at 690/735 at 77°K were thought to reflect modulation of chlorophyll protein complexes under reduced irradiances. Reduction in the chlorophyll *a/b* ratio is seen as an increase of LHC II during acclimation to reduced irradiance (Terashima and Inoue 1984). LHC II content increased in shade plants (Anderson 1980; Chow and Anderson 1987a) and low light intensity grown plants compared to sun plants and high light intensity grown plants (Lichtenthaler *et al.* 1982; Leong and Anderson 1984a; Davies *et* al, 1986; Bhaskar and Das 1987; Chow and Hope 1987; De la Torre and Burkey 1990a). In contrast, no significant changes in chlorophyll *a/b* ratio and light harvesting complex of PSII were reported in *Tradescantia* during acclimation to reduced irradiance (Chow *et al.* 1991). There is a debate about the utility of increased light harvesting complexes under reduced irradiances. Leong and Anderson (1984a) proposed that increase of LHC II may offset the low availability of longer wave length light under reduced growth irradiances, but such usefulness was disputed by other studies (Mckiernan and Baker 1991; Watanabe *et al.* 1993).

There are alterations in Chloroplast ultra structure during acclimation to suboptimal light conditions. Shade plants and the plants grown under lowered irradiances have more thylakoid membranes and less stromal volume compared to that of sun plants and high irradiance grown plants. In obligate shade plants, thylakoid membranes occupy almost entire Chloroplast diminishing the stromal volume (Anderson *et al.* 1973; Boardman

et al. 1974; Anderson 1986). Thylakoid/stromal volume and granal stacking increased in shade plants and the plants grown under lowered irradiance (Anderson 1986). Recent advances in freeze fracture studies of thylakoid membrane have made it possible to study the modulation of photosystem complexes under limiting light. PFs particles involved in thylakoid stacking increased in plants grown under reduced irradiance (McDonnel and Staehelin 1980; Staehelin 1986; Larson *et al.* 1987). Under high irradiance there is a reduction of large PFu particles corresponding to PSI and LHCPI (Green *et al.* 1988).

Photosynthetic electron transport

Emerson and Arnold defined photosynthetic unit as the number of chlorophyll molecules required to absorb light energy for the evolution of one oxygen molecule. Later it is re-defined as total chlorophyll molecules servicing P_{700} reaction centre (Björkman 1973; Boardman *et al.* 1978) or cytochrome / (Wild 1979). It was assumed by earlier workers that 1:1:1 ratio of P_{680}: cytochrome /: P_{700} remained unaltered in response to changes in irradiances but it was shown later that plants grown under reduced irradiances have altered photosynthetic stoichiometric ratios (Boardman 1977; Lichtenthaler *et al.* 1982; Leong and Anderson 1983; Hodges and Barber 1983; Leong and Anderson 1984b; Wild *et al.* 1986; Bhaskar and Das 1987; Chow and Anderson 1987b; Evans 1987b; De la Torre and Burkey 1990). In contrast, the stoichiometry of the supra molecular complexes remained unaltered in pea plants grown at reduced irradiances (Lee and Whit marsh 1989; Chow *et al* 1991). Cytochrome / content increased under high irradiance compared to low irradiance grown plants (Björkman *et al.* 1972; Boardman 1977; Leong and Anderson 1984b; Wild *et al.* 1986; Chow and Anderson 1987b; Evans 1987b; De la Torre and Burkey 1990b). Cytochrome 6, ferridoxin, plastocyanin and plastoquinone

also decreased under reduced irradiances (Boardman *et al.* 1974; Grahl and Wild 1975; Leong and Anderson 1984b). Whole chain, PSI and PSII electron transports decreased in shade plants (Boardman 1977) and plants grown under reduced irradiances (Boardman 1977; Chabot *et al.* 1979; Lichtenthaler *et al.* 1982; Hodges and Barber 1983; Leong and Anderson 1983; Davies *et al.* 1986; Bhaskar and Das 1987; Chow and Hope 1987; Davies *et al.* 1987; Anderson *et al.* 1988; De la Torre and Burkey 1990b). Photophosphorylation and components of ATP synthetase were reduced in low light grown plants (Berzborn *et al.* 1981; Leong and Anderson 1984b; Davies *et al.* 1986). Plants grown under suboptimal light had declined in photosynthetic light saturated carbondioxide fixation rates and lowered carbondioxide compensation points (Boardman 1977).

Light regulation of thylakoid proteins

Long term acclimation requires synthesis and assembly of new membrane components and degradation of existing membrane constituents. This process is regulated by light induced transcriptional, translational and post translational regulation of gene expression. (Jenkins *et al.* 1984). The light regulation of gene expression is an unique feature of photosynthetic organisms and plants. Nuclear gene coding for LHC II is regulated by light at transcriptional level in barley (Apel 1979), *Lemna* (Tobin 1981) and pea (Thompson *et al.* 1983). The light regulated expression of nuclear genes coding for plastid polypeptides seem to be controlled by transcriptional mechanisms involving binding of transfactors to their cognate cis-elements (Kuhlemeier et al 1987; Gilmartin *et al.* 1990; Thompson and White 1991; Kuhlemeier 1992). Some of the Chloroplast encoded proteins are also regulated by light (for example the psbA gene coding for D_1 protein of PSII reaction

centre). The regulation of psbA gene coding for D_1 polypeptide by light, occurs at transcriptional level (Rodermel and Bogorad 1985; Mullet and Klein 1987; Baumgartner *et al.* 1989; Klein and Mullet 1990), translation elongation site (Mullet *et al.* 1990) and at post translational site (Rochaix and Erickson 1988).

Time course of acclimation to reduced irradiance

When plants grown under one particular irradiance are transferred to another irradiance, time taken for adjustment to altered light level is called "Time Course of Acclimation". Time course of acclimation studies have proved that a number of plant species have the capacity to 'fine-tune' photosynthesis in response to changes in growth irradiance (Burkey and Wells 1991). Time course of acclimation has been studied in *Solanum* (Ferrar and Osmond 1986), *Pisum* (Chow and Anderson 1987a), *Tradescantia* (Chow *et al.* 1991), *Hordeum vulgare* (De la Torre and Burkey 1990b), *Lycopersicon* (Davies *et al.* 1986) and *Phaseolus* (Caemmerer and Farquahar 1984).

Short term acclimation to irradiance

Short term acclimation to unfavourable light conditions and temperatures involves reorganization of the components of the existing membrane and its function. Shade plants and plants acclimated to reduced irradiance when exposed to sudden high light intensities are more prone to photoinhibition in contrast to sun plants in similar circumstances. This is due to the larger photosynthetic unit size (Powles and Björkman 1982; Powles 1984). Reversible photophosphorylation of the LHC II polypeptides regulate photosynthetic unit size to protect the plants from photoinhibition (Bennett 1983; Gounaris et al 1984).

Light regulation of carbon metabolism

C_4 metabolism is essentially described as "ATP dependent" CO_2 pump (Furbank and Foyer 1988). Based on the corresponding decarboxylating enzyme, C_4 plants are classified as NADP-malic enzyme type (sugarcane, maize, sorghum) NAD-malic enzyme type (*Amaranthus, Atriplex, Cynodon)* and pep carboxykinase type (*Panicum*),(Gutierrez *et al.* 1974; Hatch *et al.* 1975; Raghavendra and Das 1976; Raghavendra and Das 1993).

Steady state levels of C_4 enzymes and their mRNAs increased several fold when dark grown plants are illuminated (Nelson *et al.* 1984; Sheen and Bogorad 1987). The runon transcription experiments in maize proved that the differential expression of Rubisco SSU genes are regulated by light in mesophyll and bundle sheath cells (Schaffner and Sheen 1991). In maize, under reduced irradiance level, mRNA of Rubisco accumulates in mesophyll and bundle sheath cells while the mRNA of C_4 enzymes are absent (Langdale *et al.* 1988). Under high light conditions, cell specific mRNA of C_4 enzymes accumulate in maize compared to that of Rubisco (Langdale *et al.* 1988). This observation is consistent with measurements of photosynthetic enzymes under high and low levels of irradiance (Hatch *et al.* 1969; Bassi and Passera 1982; Usuda *et. al.* 1985). A similar alteration in enzyme levels is reported in *Flaveria brownii* (Cheng *et al.* 1989). Leaves grown under high irradiance have high levels of pyruvate orthophosphate dikinase and phosphoenolpyruvate Carboxylase, but low levels of Rubisco and malic enzyme (Usuda *et al.* 1985).

Objectives of present work

Though the process of acclimation of photosynthesis to reduced growth irradiance has been studied by others with regard to a few species of plants, its precise nature and the time course of events leading to acclimation of Chloroplast components are still not available. This is particularly in the case of the C_4 dicotyledonous plants. Accordingly, the aim of the present work has been to document the photosynthetic acclimation process using an NAD-ME C_4 dicotyledonous plant *Amaranthus hypochondriacus* L.

1. The investigation was carried out using the following parameters to determine the changes in the components and function of thylakoid membranes. Alterations in (a) chlorophyll a/b ratio (b) fluorescence at room temperature and at 77°K (c) Fv/Fm at room temperature and at 77°K (d) SDS-polyacrylamide gel electrophoresis analysis of thylakoid polypeptides (e) P_{700} and cytochrome-/ content (f) photosynthetic capacity, were observed for the plants grown at fixed suboptimal light intensities.

2. The influence of reduced irradiance on the C_4 biochemistry through determination of levels of key enzymes involved.

3. Functional PSI and PSII reaction centre complexes were isolated and characterized using EPR spectroscopy and Fluorescence emission at 77°K to find out if the structure of reaction centres of C_4 dicot *Amaranthus* showed similarities, if any, to the reaction centre structure reported earlier for *C3* plants.

4. The progress of acclimation process with time, over a period of several days from the day of transfer of plants to different growth light regimes, was studied in *Amaranthus.*

5. Determination of the nature of expression of psbA gene coding for D_1 polypeptide of PSII reaction centre under lowered irradiance.

6. To assess the occurrence of species specificity, if any, in the acclimation syndrome, a comparison was made of the events observed in *Amaranthus hypochondriacus* L. with that of *Eleusine coracana* (L.) Gaertn. (NAD-ME monocotyledonous plant) and *Gomphrena globosa* L. (NADP-ME dicotyledonous plant).

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Seeds of *Amaranthus hypochondriacus* L. var Ag 67 were obtained from National Botanical Research Institute, Lucknow, India.

Seeds of *Eleusine coracana* (L.) Gaertn. var RP 4116 were obtained from Millet Breeder, RARS Subcentre Perumallapalli Pin: 517505, India

Gomphrena globosa L. seeds were from Horticulture Department of University of Hyderabad.

The plants of *Amaranthus*, *Eleusine* and *Gomphrena* were raised from seed in the open field under natural conditions receiving full sunlight (2000 μE $m^{-2}s^{-1}$) for forty five days. Hyderabad is geographically situated at $17.3^{o}10'$ N latitude and $78^{o}23'$ E longitude and an altitude of 542.6 m above mean sea level. These plants were denoted as high light (H) plants. Two other batches of plants were grown at one third of natural irradiance (650 μE $m^{-2}s^{-1}$) and one tenth of natural irradiance (200 μE $m^{-2}s^{-1}$) and were called as low light plants L_1 and L_2 respectively. Reduced irradiances were obtained by screening natural sunlight through appropriate wooden reapers held above the canopy. Forty five day old plants were used for experiments. The photoperiod was approximately twelve hours. The plants were watered twice a day. The day temperature varied from 30° to 40^{o}C. The night temperatures varied from 12° to 25^{o}C.

The growth of plants under different light regimes was as per the following schedule.

S.No.	Level of growth light prior to transfer	Level of growth light on transfer	Designation of plants.
1)	High light ($2000\ \mu E\ m^{-2}s^{-1}$)	Control (fixed irradiance)	**H**
2)	Low light ($650\ \mu E\ m^{-2}s^{-1}$)	Control (fixed irradiance)	L_1
3)	Low Light ($200\ \mu E\ m^{-2}s^{-1}$)	Control (fixed irradiance)	L_2
4)	H	L_1	
5)	H	L_2	$H \rightarrow L_2$
6)	L_1	**H**	$L_1 \rightarrow H$
7)	L_2	**H**	$L_2 \rightarrow H$

Bundle sheath and mesophyll thylakoid membranes

Bundle sheath and mesophyll thylakoid membranes were isolated by the procedure of Vainstein *et al.* (1989). The leaves were ground in a suspension buffer containing 0.6 M sorbitol, 10 mM HEPES-KOH (pH 7.5) and 2 mM $CaCl_2$. The suspension was filtred through two layers of miracloth and 80 μM nylon mesh. The filtrate was pelleted by centrifugation at 5,000 rpm. The pellet containing mesophyll chloroplasts were suspended in 10 mM HEPES-NaOH (pH 7.5) and 2 mM $CaCl_2$ for ten minutes and then centrifuged at 7,000 rpm again for 10 minutes. The pellet containing mesophyll thylakoid membranes was suspended in suspension buffer (10 mM HEPES-KOH (pH 7.5) and 0.3 M sorbitol).

The residue **from** the grinding and filtration steps described above was resuspended in suspension buffer and blended for one additional minute in a razor blender and then filtered through miracloth. This process was repeated four times, and the homogenate was filtered through 20 μm and 35 μm mesh sieves. The filtrate was filtered through a 80 μm nylon mesh net. The final residue was blend for 5 seconds in a razor blade blender. Bundle sheath chloroplasts were pelleted at 3,000 rpm. Bundle sheath chloroplasts were suspended in osmotic shock medium (10 mM HEPES-NaOH (pH 7.6) and 2 mM $CaCl_2$) and then centrifuged at 7,000 rpm for 10 minutes. The pellet containing bundle sheath thylakoid membranes was suspended in suspension buffer. Both mesophyll and bundle sheath thylakoid membranes were used for electron transport studies and for determining polypeptide profile by SDS- PAGE.

Carbon fixation

CO_2 assimilation rates and stomatal conductivity were measured using open gas exchange system (ADC portable IRGA comprising LCA-2 analysers, ASU/ ME, PLC-B C-(B) and DL-1). Leaves were fixed in PLC-B cuvette with neoprene rubber gas belts. The leaf cuvette contains a humidity sensor (solid state) positioned in exhaust air, an air temperature sensor (thermistor) and a PAR sensor. (Selenium cell consisting of filters). All the electronics associated with the sensors were located within the cuvette handle which was connected to the LCA by a cable. Measured flow of air was drawn above **four** metres from ground level using ASU/MF by mounting it on mast. CO_2 assimilation and stomatal conductivity were determined by differential mode. Ten values were taken. The data was **fed** into **IBM** PC **with a printer** attached to it.

Chlorophyll estimation

Chlorophyll was estimated by the procedure of Arnon (1949). 100 mg of leaf sample was ground in 10 ml of 80% acetone and was centrifuged at 2,000 rpm for five minutes and optical density was read at 663 nm and 648 nm. Chlorophyll concentration was calculated by the following formulae.

chlorophyll $a + b = 20.2 \times A_{645} + 8.02 \times A_{663}$

chlorophyll $a = 12.7 \times A_{663} - 2.69 \times A_{645}$

chlorophyll $b = 22.9 \times A_{645} - 4.68 \times A_{663}$

Cytochrome *f* estimation

Cytochrome / levels were determined by the procedure of Bendall *et al.* (1971). Bundle sheath and mesophyll thylakoid membranes were suspended in buffer containing 10 mM HEPES-KOH (pH 7.8), 10 mM NaCl, 30 mM sodium ascorbate and 0.4 M sucrose and centrifuged at 16,000 rpm for 5 minutes. The pellet was suspended in buffer (10 mM HEPES (pH 7.8), 10 mM NaCl and 30 mM ascorbate) and centrifuged at 16,000 rpm. for 5 minutes. The pellet was resuspended in buffer containing 20 mM TRICINE-KOH (pH 7.8) and 10 mM NaCl. 20 μg of chlorophyll was taken in 1 ml suspension buffer in 1.5 ml quartz cuvettes. Base line correction was performed in dual beam dual wave length spectrophotometer (model 557, Hitachi). Then ferricyanide oxidized minus hydroquinone reduced spectra was recorded from 450 nm to 550 nm. The cytochrome / concentration was calculated using molar extinction coefficient of 19.7 mM cm^{-1}.

Photosynthetic electron transport

Photosynthetic electron transport measurements were performed according to the procedure of De la Torre and Burkey (1990b). Assay buffer contained 50 mM HEPES-NaOH (pH 7.8) and 0.1 M sorbitol. Whole chain electron transport rates from $H_2O \rightarrow$ methyl viologen (MV) were measured polarographically at 25 °C as oxygen uptake using a Clark-type electrode in Gilson oxygraph. The uncoupled whole chain electron transport was measured in a assay buffer consisting of 1 mM NH_4Cl, 1 mM NaN_3, 0.1 mM MV and 5 μg chlorophyll/ml thylakoid membranes. The PSI electron transport rates (Asc/DCPIP$\rightarrow$ MV) were measured by monitoring oxygen uptake at 25 °C using Gilson oxygraph. The PSI electron transport reaction mixture contained assay buffer, 1 mM NaN_3, 2 μM DCMU, 2 mM sodium ascorbate, 0.1 mM MV 10 mM sodium ascorbate and 5 μg chlorophyll/ml thylakoid membranes.

PSII activity was measured as DCPIP reduction at 590 nm using molar extinction coefficient for DCPIP which is 18.9 mM^{-1}cm. The reaction mixture contained assay buffer, 1 mM NH_4Cl, 0.5 mM BQ, 30 μM DCPIP and 2 μg chlorophyll/ml of thylakoid membranes.

Electron spin resonance spectra

PSI particles were suspended in PSI isolation buffer (50 mM TRICINE-NaOH pH 7.8, 300 mM sucrose and 20 mM $MgCl_2$) and EPR spectra was measured for PSI complex at 77°K in EPR spectrometer (model JOEL.FB-3). Manganese signal in PSII complex was measured in isolation buffer (20 mM MES buffer pH 6.0, 300 mM sucrose and 10 mM NaCl). Spectra were taken at both room temperature and at liquid nitrogen

temperatures. Parameters used are mentioned in legends.

Enzymes

Phosphoenol pyruvate Carboxylase (E C 4.1.1.3).

Phosphoenol pyruvate-carboxylase was assayed according to the procedure of Iglesias and Andrea (1989). 1 gm of leaf was homogenized in 4 ml of extraction buffer (10 mM Tris-HCl pH 7.3, 10 mM $MgCl_2$, 2 mM K_2HPo_4, 1 mM EDTA, 20% glycerol, 10 mM 2-mercaptoethanol and 2 g solid PVP), filtered through cheese cloth and centrifuged at 12,000× g for 15 minutes.

The enzyme activity was determined spectrophotometrically at 30°C by following NADH oxidation at 340 nm using Shimadju spectrophotometer by coupling the carboxylase reaction with malate dehydrogenase. The standard assay medium contained 50 mM Tris-HCl pH 8.0, 5 mM $MgCl_2$, 0.15 mM NaOH, 10 mM $NaHCO_3$, 2 units malic dehydrogenase, 4 mM PEP and enzyme in total volume of 1 ml. NADH concentration was determined using molar extinction coefficient for NADH. (ϵ_{340} = 6.22 x 10^3 $cm^{-1}s^{-1}$).

NADP-Malic enzyme (EC 1.1.82).

NADP-ME was assayed according to the procedure of Iglesias and Andrea (1989). One gram of leaf was homogenized in 4 ml of extraction buffer consisting of HEPES-NaOH pH 7.4, 5 mM $MgCl_2$, 2 mM EDTA, 10 mM 2-mercaptoethanol and solid PVP. The extract was centrifuged at 12,000 ×g for 15 minutes and the supernatant was used as crude enzyme preparation.

NADP-ME activity was assayed spectrophotometrically at 30°C following the NADPH

production at 340 nm. The standard assay medium had 50 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$, 0.5 mM NADP, 4 mM malate and malic enzyme in a final volume of 1 ml.

NAD-Malic enzyme: (EC 1.1.1.39).

NAD-ME was assayed according to the procedure of Hatch *et al.* (1982). One gram of leaf was homogenized in 4 ml of extraction buffer (50 mM HEPES-NaOH pH 7.6, 5 mM $MgCl_2$, 10 mM β mercaptoethanol and 0.2% BSA) filtered through cheese cloth and centrifuged at 12,000 rpm for 10 minutes.The supernatant was used for enzyme estimation.

The enzyme was assayed in assay buffer consisting of 25 mM HEPES-NaOH pH 7.6, 1 mM EDTA, 10 mM β-mercaptoethanol, 5 mM $MnCl_2$, 0.2 mM NAD and 5 mM fructose 1,6 bisphosphate. The reaction was initiated using 5 mM malate. The NAD reduction was monitored at 340 nm. The enzyme concentration was calculated using molar extinction coefficient for NAD. (6.22x 10^3 cm^{-1} s^{-1}).

Pyruvate orthophosphate dikinase: (EC 2.7.9.1).

Crude enzyme was prepared and assayed according to the procedure of Usuda *et al.* (1984). 1 gm of leaf was homogenized in 4 ml of extraction buffer (50 mM HEPES-KOH pH 8.0, 5 mM $MgCl_2$ and 5 mM DTT) filtered through cheese cloth and centrifuged at 12,000 rpm for 10 minutes. The supernatant was used for assaying the crude enzyme.

Activity was determined in 1 ml cuvettes containing 50 mM HEPES-KOH pH 8.5, 5 mM DTT , 8 mM $MgSO_4$, 10 mM $NaHCO_3$, 1 mM ATP, 1 mM glucose-6phosphate,

5 mM $(NH_4)_2SO_4$, 5 mM pyruvate, 2 mM Pi, 0.2 mM NaOH, 1 unit of malate dehydrogenase and approximately 0.2 units of phosphoenol pyruvate Carboxylase. Activity was followed by the decrease in absorbance at 340 nm due to oxidation of NADH by OAA formed from phosphoenol pyruvate via phosphoenol pyruvate Carboxylase.

Malate dehydrogenase (EC 1.1.1.37).

The crude enzyme was assayed according to the procedure of Hatch *et al.* (1982). One gram of leaf was homogenized in 4 ml of extraction buffer (50 mM HEPES-NaOH pH 7.6, 5 mM $MgCl_2$, 10 mM β mercaptoethanol and 0.2% BSA) filtered through cheese cloth and centrifuged at 12,000 rpm for 10 minutes.

The enzyme was assayed in the assay buffer consisting of 25 mM HEPES-NaOH pH 7.6, 1 mM EDTA, 10 mM β-mercaptoethanol, 0.2 mM NaOH and 40 mM OAA. The NADH oxidation was monitored at 340 nm. The enzyme concentration was calculated using the molar extinction coefficient for NADH. $(\epsilon=6.22\times10^3 cm^{-1}s^{-1})$.

NADP-Malate dehydrogenase (EC 1.1.1.82).

The enzyme assay was done spectrophotometrically by monitoring the oxidation of NAD PH at 340 nm (Kanai and Edwards 1973). One gram of leaf was ground in extraction medium (50 mM TRICINE-KOH pH 8.0, 5 mM $MgCl_2$ and 5 mM DTT).

An aliquot of the enzyme was pre-incubated in 25 mM DTT in a volume of 0.1ml, for 15 minutes at room temperature. The enzyme extract (5 *fig* chlorophyll) was activated by incubating in reaction mixture (1 ml) containing 50 mM TRICINE-KOH (pH

8.0), 0.2 mM NADPH and DTT. The reaction was started by adding OAA to a final concentration of **3** mM.

Aspartate (2: oxoglutarate) amino transferase (EC 2.6.11).

The enzyme was assayed according to the procedure of Edwards and Guiterrez (1973). One gram of leaf tissue was ground in 4 ml of grinding buffer (50 mM HEPES-NaOH pH 7.6, 0.33 M sucrose, 10 mM $MgCl_2$, 10 mM β-mercaptoethanol and 100 mM PVP) filtered through cheese cloth and centrifuged at 10,000 rpm. The supernatant was used to assay the enzyme.

The enzyme was assayed in the medium consisting of 50 mM HEPES-NaOH pH 7.8, 50 mM aspartate, 2 units of malate dehydrogenase, 25 μg of pyridoxal phosphate, 0.2 mM NADH and 25 mM α-ketoglutaric acid. The NADU oxidation was monitored at 340 nm and the enzyme was estimated using the molar extinction coefficient for NADH.

Alanine (2: oxoglutarate) amino transferase (EC 2.6.12)

The enzyme alanine amino transferase was assayed according to the procedure of Edwards and Guiterrez (1973). The isolation medium and crude enzyme preparation procedure was identical to the preparation of crude aspartate amino transferase. The enzyme was assayed in the medium consisting of 50 mM HEPES-NaOH pH 7.8, 25 mM alanine, 2 units of lactate dehydrogenase, 25 μg of pyridoxal phosphate, 0.2 mM NaOH and 25 mM α-ketoglutaric acid. The NADH oxidation was monitored at 340 nm and the enzyme was estimated using the molar extinction coefficient for NADH.

Fluorescence measurements

Fluorescence emission spectra for whole leaves, thylakoid membranes and supra molecular complexes of thylakoid membrane were recorded using Hitachi fluorescence spectrophotometer (model 4010). The spectra were recorded at 490 nm excitation wavelength, using 5 nm as excitation bandpass, 5 nm as emission band pass and instrument response 2. The fluorescence emission spectra for PSII complex and LHCII were recorded at 440 nm excitation wavelength. Fluorescence was recorded at 77 ^{o}K using liquid nitrogen attachment and circulating nitrogen gas through the inlet and out let of the low temperature attachment of the Hitachi fluorescence spectrophotometer. The instrument parameters were similar to the parameters used for recording room temperature fluorescence emission spectra.

Measurements of fluorescence transients at room temperature

The fluorescence transients are measured using chlorophyll fluorometer model Sf-30 (Richard Brancker, Canada). The leaf was dark adapted for 15 minutes and then illuminated at 670 nm by high intensity light emitting diodes which are coaxially mounted to a photodiode sensor. The photocell uses the LED as a lens to collect the fluorescent radiation from the plant leaf. The light was filtered before detection and amplified for further processing by the control unit. The fluorescent activity is related to photosynthetic energy conversion. The ground level fluorescence was called F_o. the peak of Kautsky's signal was fluorescence maxima or F_m. Variable fluorescence F_v is obtained by subtracting F_o from F_m. The fluorescence induction curves were plotted by connecting the instrument to an IBM pc.

Measurement of fluorescence transients at 77°K

Fluorescence kinetics were measured at 77°K by the procedure of Powels and Björkman (1982) in Hithachi fluorescence spectrophotometer having low temperature attachment. The leaves were kept for ten minutes in darkness before taking fluorescence measurements. The leaves were maintained in the dewar flask at room temperature for five minutes. Then liquid nitrogen was added to the flask. The bandpass was adjusted to pass 13 μ mol $m^{-2}s^{-1}$ of light intensity. Time scan was performed for two minutes. The value obtained was F_o. Then the intensity of exciting light was increased to 130 μ mol $m^{-2}s^{-1}$ to get F_m (Fluorescence maxima). F_v was obtained by subtracting F_o from F_m.

Supramolecular complexes

PSI complex.

PSI particles were isolated and purified according to the procedure of Mullet *et al* (1980) with slight modifications. The leaves were homogenized in a buffer containing 0.4 M sorbitol, 10 mM EDTA and 50 mM TRICINE-KOH pH 7.8, filtered the slurry through four layers of cheese cloth and centrifuged at 10,000 x g for 5 minutes. The pellet was resuspended in a medium containing 0.5 M sorbitol, 5 mM EDTA, 50 mM TRICINE-NaOH pH 7.8 and centrifuged at 10,000 x g for 5 minutes. The pellet was resuspended in distilled water to give chlorophyll concentration of 0.8 mg/ml. The destacked thylakoid nembranes were solubilized by the addition of 20% Triton X-100 to a final concentration of 0.6-0.7% and incubated for 30 min at 20°C with constant stirring. The contents were centrifuged at 42,000 g for 30 min in Beckman VT;70 rotor. Eight **ml of** the supernatant **was** loaded on **0.1 M** to 1.0 **M linear sucrose** gradient made **in** 5 **mM TRICINE-KOH,**

pH 7.8 and 0.8% Triton-X-100 underlaid with 2 M sucrose cushion and centrifuged in Beckman SW60 rotor at 50,000 rpm for five hours. The lower most band was collected carefully and was stored at -80°C for further use. PSI core and LHCPI were separated according to the procedure of Lam *et al.* (1984). PSI complex was solubilized in Triton-X-100 and then centrifuged on a linear gradient of 0.1 to 1 M sucrose gradient at 50,000 rpm for five hours. The two bands were collected from the gradient and stored at -80°C for further use.

PSII complex

The PSII complex was prepared from thylakoid membranes by solubilizing with a high concentration of Triton-X-100 (Ikeuchi *et al.* 1985). The chilled leaves were homogenized in extraction buffer (0.3 M sucrose, 50 mM Tris-HCl (pH 7.8), 20 mM NaCl, 5 mM $MgCl_2$ and 0.2% BSA). The slurry was filtered through four layers of cheese cloth and centrifuged at 3,000 rpm for 5 minutes. The pellet so obtained was washed in suspension buffer (50 mM HEPES-NaOH buffer (pH 7.5), 5 mM $MgCl_2$ and 0.2% BSA) and solubilized in Triton-X-100 (25 mg per mg chlorophyll). The suspension was incubated at 4°C for 30 minutes with occasional stirring and centrifuged at 40,000×g for 30 minutes. The pellet obtained was suspended in the suspension buffer and Triton-X-100 (5 mg/mg chlorophyll) was added and centrifuged at 40,000×g for 30 minutes. The PSII reaction centre was isolated from PSII particles as described by Nanba and Satoh (1987). The PSII particles (1 mg chlorophyll/ml) were further treated with 4% (w/v) Triton-X-100 in 50 mM Tris-HCl (pH 7.2) for 1 hour at 4°C with occasional stirring. The solubilized PSII reaction centre complex was centrifuged at 1,00,000×g for 1 hour at 4°C. The supernatant was fractionated on ion exchange DEAE cellulose column. PSII reaction centre was eluted using 30 mM to 200 mM NaCl gradient.

LHCII complex

The light harvesting complex was isolated by the method of Butler and Kuhlbrandt (1987). The thylakoid membrane pellet was resuspended in 50 ml of 0.5 mM EDTA and 0.1 M sorbitol and 50 mM TRICINE-NaOH, pH 7.5. The thylakoid membranes were reisolated by centrifuging at 7,000 rpm for 7 minutes. The pellet was washed and resuspended at the concentration of 0.5 mg chlorophyll/ml in distilled water and the pH was then adjusted to 7.5 using 1 M $(CH_3)_4NOH$. Triton-X-100 was added at a concentration of 0.5% (from stock solution of 20% Triton-X-100) and the pH was readjusted to 7.5. The suspension was centrifuged at $1{,}00{,}000 \times g$ for 30 minutes in Beckman VTi 70 rotor. The supernatant was layered over a linear gradient (0.05 M to 0.5 M) of sucrose prepared in suspension buffer (50 mM TRICINE NaOH pH 7.5 and 0.05% Triton-X-100) and was centrifuged at 26,000 rpm for 5 hours in Beckman SW28 rotor. The major band at the top of the gradient was removed and adjusted to pH 7.5. 10 mM $MgSO_4$ was then added and the solution was stirred for 20 minutes at 25°C. Four ml aliquot was pelleted through 0.2 M sucrose cushion. LHCII particles were stored at -80°C.

Oxygen evolution

Oxygen evolution rates were determined using leaf disc oxygen electrode. (Hansatech LD2 attached to personal computer). The leaves were kept for light activation (50 μ moles Q $m^{-2}s^{-1}$) for one hour. The leaf was dark adapted for 5 minutes and the measurements were done on 10 cm^2 leaf area. Red light emitting diodes were used for determining oxygen evolution rates at photon fluence rates of 855 μ mol quanta $m^{-2}s^{-1}$.

P_{700} estimation

P700 content was determined by the procedure of Hiyama and Ke (1972). Ferricyanide oxidized and ascorbate reduced difference spectra was taken from 650 nm to 750 nm. Samples of equal chlorophyll concentration were placed in identical cuvettes. After recording the base line, 25 μM $K_3Fe(CN)_6$ was added to sample cuvette and 0.5 mM sodium ascorbate was added to reference cuvette and was allowed to equilibrate prior to recording the spectra. Reversible absorbance changes were again recorded by rereducing the oxidized sample. P700 content was calculated using an absorption coefficient of 64 $mM^{-1}cm^{-1}$. (Hiyama and Ke 1972).

Protein estimation

Protein estimation was done according to Lowry et al (1951).

SiMo of PSII reaction centre.

Photochemical competence of reaction centre was assayed by photoinduced electron transport from DPC to Simo at 25°C (was seen as increase in optical density at 600 nm) using Hitachi 557 dual wavelength dual beam spectrophotometer according to the procedure of Barber et al (1987). Reaction centre was diluted to 0.7 μg chlorophyll/ml in 60 mM Tris-HCl (pH 8.5), 0.25% Triton-X-100. 43 μg/ml diphenyl carbazide (DPC) was added and incubated for 2 minutes. SiMo was then added at a concentration of 2 mg/ml. Photomultiplier was shielded using red cutoff filter. Activity was calculated using extinction coefficient for SiMo (4.8 $mM^{-1}cm^{-1}$).

SDS- PAGE of thylakoid polypeptides

Thylakoid membranes, PSI particles, PSII complex, LHC II complex were subjected to polyacrylamide gel electrophoresis using the procedure of Laemmli (1970). Polypeptides were separated on 12% resolving gel (pH 8.5). 5% stacking gel acrylamide (pH 6.8) was used for stacking the proteins. The proteins were separated at 100 v current for $5\frac{1}{2}$ hours. Thlakoid membranes equivalent to $50\mu g$ chlorophyll was loaded in individual lanes in figure 5.1. PSI preparation equivalent to to 10 μg chlorophyll was loaded per lane in figure 1.1. LHCII preparation equivalent to 5 μg chlorophyll was loaded per lane in figure 5.3.

Molecular biological techniques

Source of clones

Heterologous Chloroplast DNA probe pTB28 from *Nicotiana tabacum* coding for D_1 polypeptide of PSII reaction centre complex was obtained in the form of stab cultures of transformed bacteria. The clone was a kind gift from Prof. M. Sigiura, Japan.

Amplification and purification of plasmid DNA

The stab culture containing transformed bacteria (plasmids containg psbA gene) were multiplied by inoculating colonies from stab culture into 50 ml of Luria Broth (10 g of tryptone, 5 g yeast extract, 10 g NaCl per litre medium and pH adjusted to 7.5 with NaOH) medium containing 35 $\mu g/ml$ ampicillin Sulfate in 100 ml flask. The culture was incubated at 37^oC for 2.5 hours on a rotary shaker at 200 rpm. Plasmid DNA was purified according to the alkaline lysis method (Sambrook *et al.* 1989). The culture was pelleted

at 4,000 rpm for 10 minutes. The pellet was suspended in solution I (50 mM glucose, 25 mM Tris-HCl pH 8.0, 10 mM EDTA). Then freshly prepared solution II (0.2% NaOH and 1% SDS) was added and cyclomixed. The solution III (5 M with respect to potassium and 3 M with respect to acetate) was added and kept on ice for 5 minutes. The contents were pelleted at 12,000 rpm for 10 minutes. Supernatant containing plasmid DNA was extracted with phenol, CHISAM (chloroform:isoamyl alcohol 24:1), precipitated by distilled chilled 100% ethanol and washed once with 70% ethanol by centrifuging at 12,000 rpm. The pellet was vacuum dried and then dissolved in 1× TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0).

Preparation of total RNA from *Amaranthus hypochondriacus* L. Leaves

RNA was isolated according to the procedure of Freeman *et al.* (1990). 2 gms of plant material was frozen in liquid nitrogen and ground to a fine powder in mortar and pestle. 4.5 ml of NTES buffer (0.1 M Tris-HCl pH 7.5, 0.1 M NaCl, 1 mM EDTA and 1% SDS) and 3 ml of 1:1 ratio of phenol and CHISAM (chloroform isoamylalcohol in the ratio of 24:1) was added and incubated at 45°C. The extract was centrifuged at 10,000 rpm. The supernatant was removed and the pellet was re-extracted with NTES buffer. The aqueous phase containing RNA was extracted with Tris buffered phenol. Nucleic acids were precipitated by adding 2 volumes of ethanol and 0.1 volume of 3 M sodium acetate. The precipitate was washed with 70% ethanol and then suspended in sterile water. Equal volumes of 4 M LiCl was added and RNA was selectively precipitated at 4°C over night and pelleted at 10,000 rpm for 10 minutes. The pellet was resuspended in sterile water. RNA was precipitated with 0.1 ml of 2 M sodium acetate and 2 ml ethanol. The pellet was washed with 70% ethanol and suspended in lx TE (10 mM Tris, 1 mM EDTA, pH 8.0).

Electrophoresis of RNA

Denaturing agarose gel electrophoresis of RNA was performed according to the procedure of Sambrook *et al.* (1989) 1.2% agarose gel was prepared. For RNA denaturation, to 5μg of RNA, 2 μl of 5× gel running buffer (0.1 M MOPS buffer pH 7.0, 50 mM sodium acetate, 5 mM EDTA pH 8.0), 3.5 μl of formaldehyde and 10 μl of formamide were added. After incubating at 65° for 15 minutes, 2 μl of gel loading buffer (50% glycerol, 1 mM EDTA pH 8 and xylene cyanol) was added to denatured RNA and electrophoresis was performed for 6 hours in 1X formaldehyde gel running buffer.

Northern blotting of RNA to nylon membrane

Denatured RNA from agarose gels were blotted on to the nylon membrane (Gene screen Du point) according to manufacturers (LKB 2016 Vacellgene) instructions by using Vacugene vacuum blotting apparatus (LKB 2016 Vacugene pharmacia). The gels were denatured in alkaline solution (1 mM NaOH and 1 mM NaCl) and neutralized in buffer containing 100 mM Tris-HCl pH 7.4 for 5 minutes each. The transfer was allowed to proceed for two hours at 40 M bar pressure in 20X SSC (1X SSC=150 mM NaCl and 15 mM sodium citrate pH 7.5). The blots were washed in 2X SSC, air dried and baked at 80°C under vacuum for two hours.

Labelling of probes

The cloned fragment of 7.3 kb insert was digested using Bgl I restriction endonuclease Genei, India). The insert DNA along with vector was denatured by heating at 95°C for minutes, snap cooled on ice for 5 minutes and labelled using ^{32}P dCTP (deoxy cytidine 5' triphosphate) by the random-prime method of Feinberg and Vogelstein (1983 & 1984)

using the oligo labelling kit from Pharmacia according to suppliers instructions. The probe was labelled in 50 μl reaction mixture containing 50 ng of denatured probe DNA, 10 μl of reaction mixture, 5 μl of ^{32}P dCTP, 1 μl Klenow enzyme and sterile distilled water to make up the final volume. The reaction mixture was incubated at 37°C for $2\frac{1}{2}$ hours. The labelled probe was again denatured by heating for 5 minutes at 95°C and subsequent snap cooling on ice.

Hybridization

Northern blots were prehybridized in sealed polythene bag containing Prehybridization solution (7% SDS, 1% BSA and 500 mM Na_2HPO_4, pH 7.2 and 20 μg/ml of sheared, denatured salmon sperm DNA). The air bubbles were removed and Prehybridization proceeded for four hours at 65°C in Haaku shaker water bath. Hybridization was done by injecting the labelled probe into fresh Prehybridization mixture. The bag was resealed and incubated for at least 18 hours at 65°C. Following hybridization, the membrane was washed for 30 minutes in each of 3X SSC and 0.1% SDS, 1X SSC and 0.1% SDS and finally in 0.1X SSC and 0.1% SDS solution. Washing was done three or four times till background count was eliminated. The membrane was air dried by blotting dry between layers of tissue papers and enclosed in saran wrap. Autoradiograms were developed at -70°C by exposing the membrane to Indu X-ray films. The film was developed with KODAK developer for three minutes, followed by stop-bath (1% acetic acid solution) for 1 minute, fixed with KODAK fixer for 3 minutes, washed in running water and air dried. The autoradiograms were scanned for band intensities with Xenith soft laser scanning densitometer (Biomed instruments USA).

List of Chemicals

The following chemicals are obtained from Sigma Chemical Co., St. Louis, U.S.A.: alanine, aspartate; benzoquinone, BSA, DCMU, diphenyl carbazide, α-ketoglutaric acid, lactate dehydrogenase, malate dehydrogenase, MES, OAA, phosphoenol pyruvate, phosphoenol pyruvate Carboxylase, PVP, pyridoxal phosphate, Tris, TRICINE.

The following chemicals are obtained from Fluka chemie AG, Switzerland: MV, sodium ascorbate, SDS and Triton-X-100.

The following chemicals were of molecular biology grade which were obtained from Sigma Chemical Co., St. Louis, U.S.A.: EDTA, formaldehyde, formamide, LiCl, MOPS, Na_2HPO_4, sodium acetate, sodium citrate, salmon sperm DNA, xylene cyanol.

Klenow enzyme was obtained from Pharmacia Bromma, Sweeden.

The rest of the chemicals used were of analytical grade manufactured in India by different firms.

% decrease of control and % increase of control are calculated as follows in tables.

$$\frac{H \text{ value} - L_1 \text{ value}}{H \text{ value}} \times 100$$

$$\frac{H \text{ value} - L_2 \text{ value}}{H \text{ value}} \times 100$$

$$\frac{H \text{ value} - L_1 \text{ value}}{L_1 \text{ value}} \times 100$$

$$\frac{H \text{ value} - L_2 \text{ value}}{L_2 \text{ value}} \times 100$$

RESULTS

Photosystems

The PSI complex isolated from *Amaranthus* consists of 83/82 kDa het erodimer but seen as 66 kDa heterodimer during SDS-PAGE of PSI polypeptides, 23 kDa, 18 kDa and two lower molecular weight polypeptides (Figure 1.1). The function of reaction centre was assayed for P_{700} reduction in the presence of potassium ferricyanide and sodium ascorbate. Fluorescence emission spectra at 77°K showed signal at 720 nm (Figure 1.2). The PSI complex showed EPR signal at g=1.99 at 77°K (Figure 1.3).

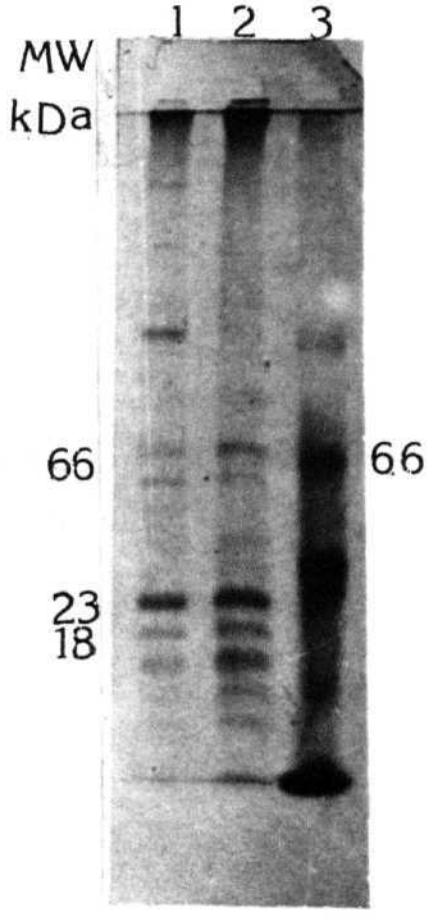
The isolated PSII complex was loaded on to a DE-52 column. The reaction centre was eluted using 0 to 200 mM $MgSO_4$ (Figure 1.4). The fractions with maximum SiMo photoreduction were pooled and concentrated by ultrafiltration. PSII reaction centre showed fluorescence emission peak at 685 nm at 77°K (Figure 1.5). Multiline spectra at *9=2* was observed for the reaction centre (Figure 1.6).

Growth of plants

L_1 and L_2 plants showed reduced growth in *Amaranthus, Eleusine* and *Gomphrena* (Figures 2.1a, 2.2a and 2.3a). Anthocyanin pigment content in the leaves of *Amaranthus* was directly proportional to the growth light available for the plants (Figure 2.1a). The Pigments disappeared in // → L_1 plants while the pigments reappeared in L_1 → *H* plants (2.1b). The relative growth of *Eleusine* and *Gomphrena* for transfer experiments are shown in figures 2.2b and 2.3b.

Chlorophyll *a/b* ratio decreased in *L* and L_2 plants of *Amaranthus, Eleusine* and *Gomphrena. Gomphrena* plants grown at reduced irradiances showed least reduction in the chlorophyll *a/b* ratio compared to *Amaranthus and Eleusine* grown at similar

Figure 1.1. Polypeptide profile of PSI complex isolated from *Amaranthushypochondriacus*. L Lanes 1 and 2: PSI complex from two separate preparations Lane 3: marker proterns.


1
2
3
MW
kDa
66
23
18
66

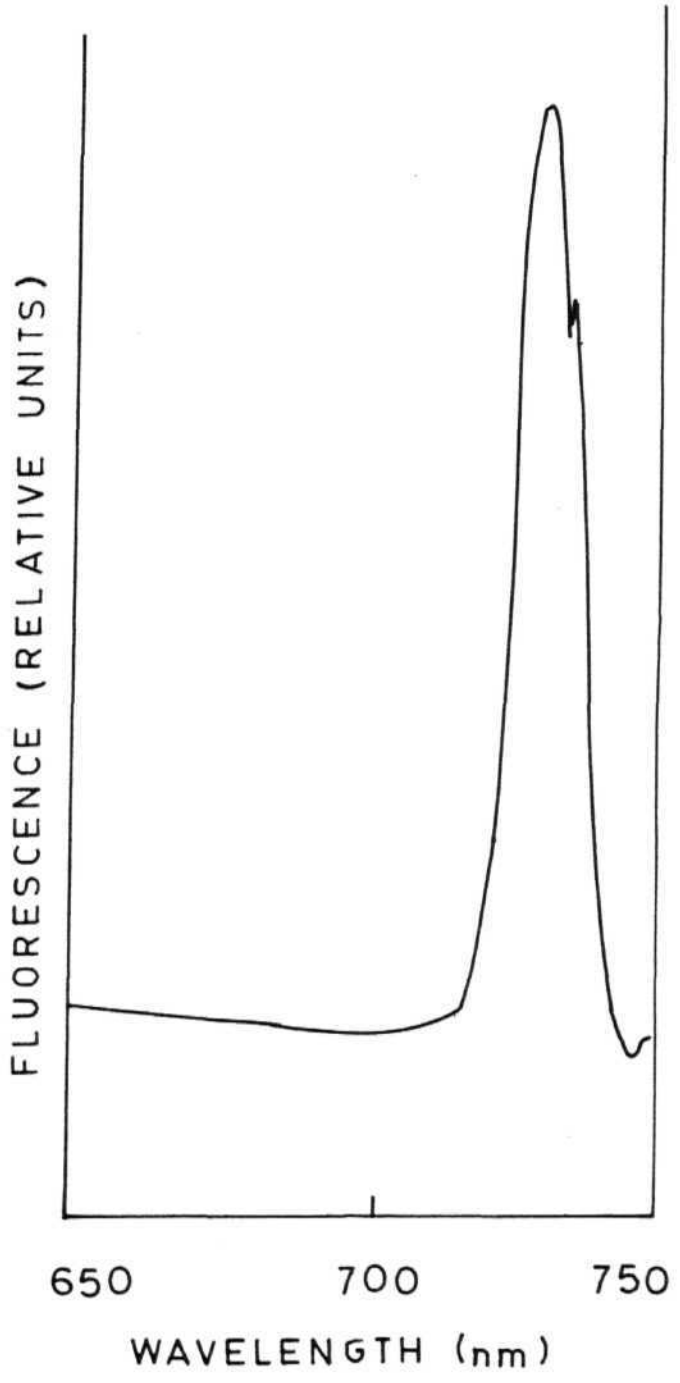
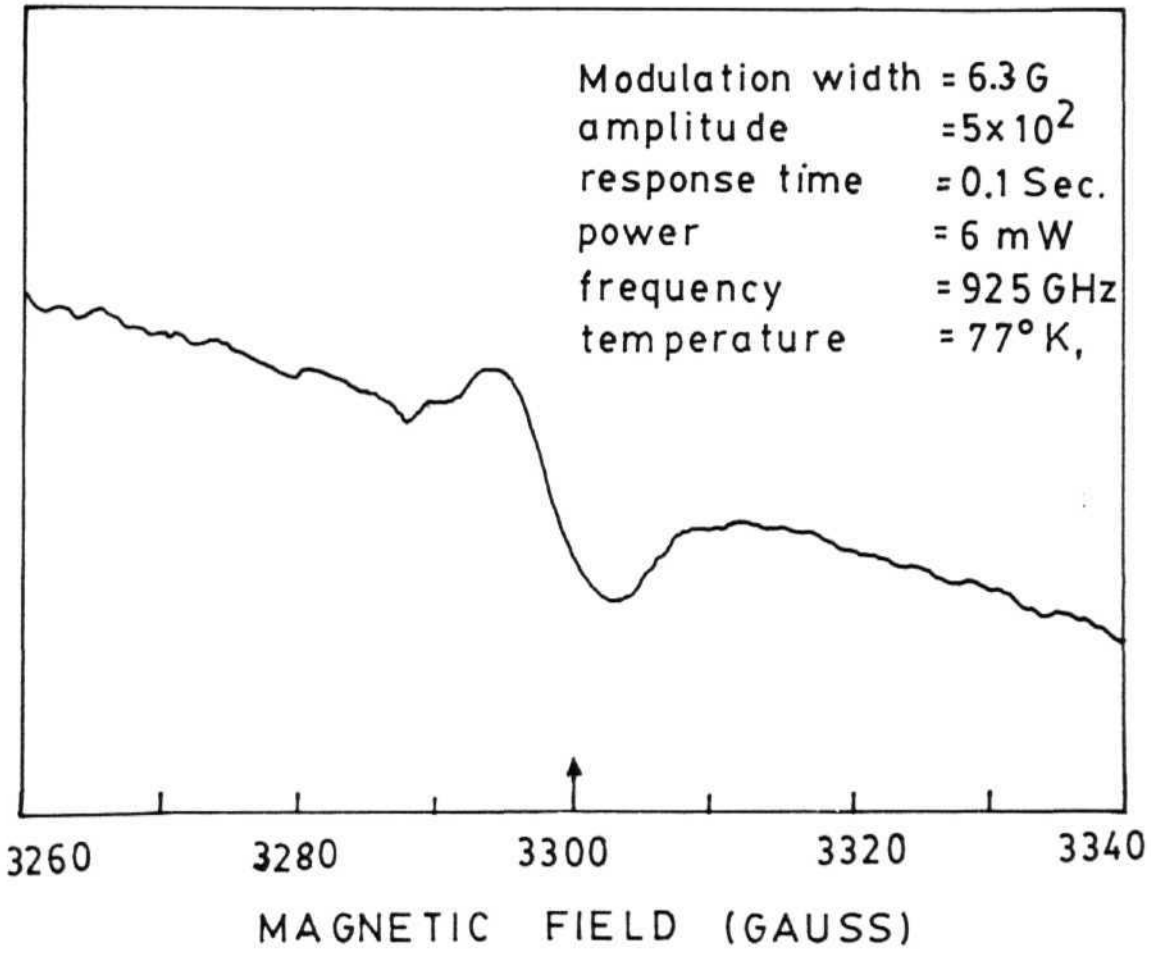


Figure 1.2: Fluorescence emission spectra of PSI complex at 77°K.
Excitation wavelength: 490nm.

Figure 13. **EPR Spectra of PSI reaction centre complex. From Amaranthus hypochondriacus L.**



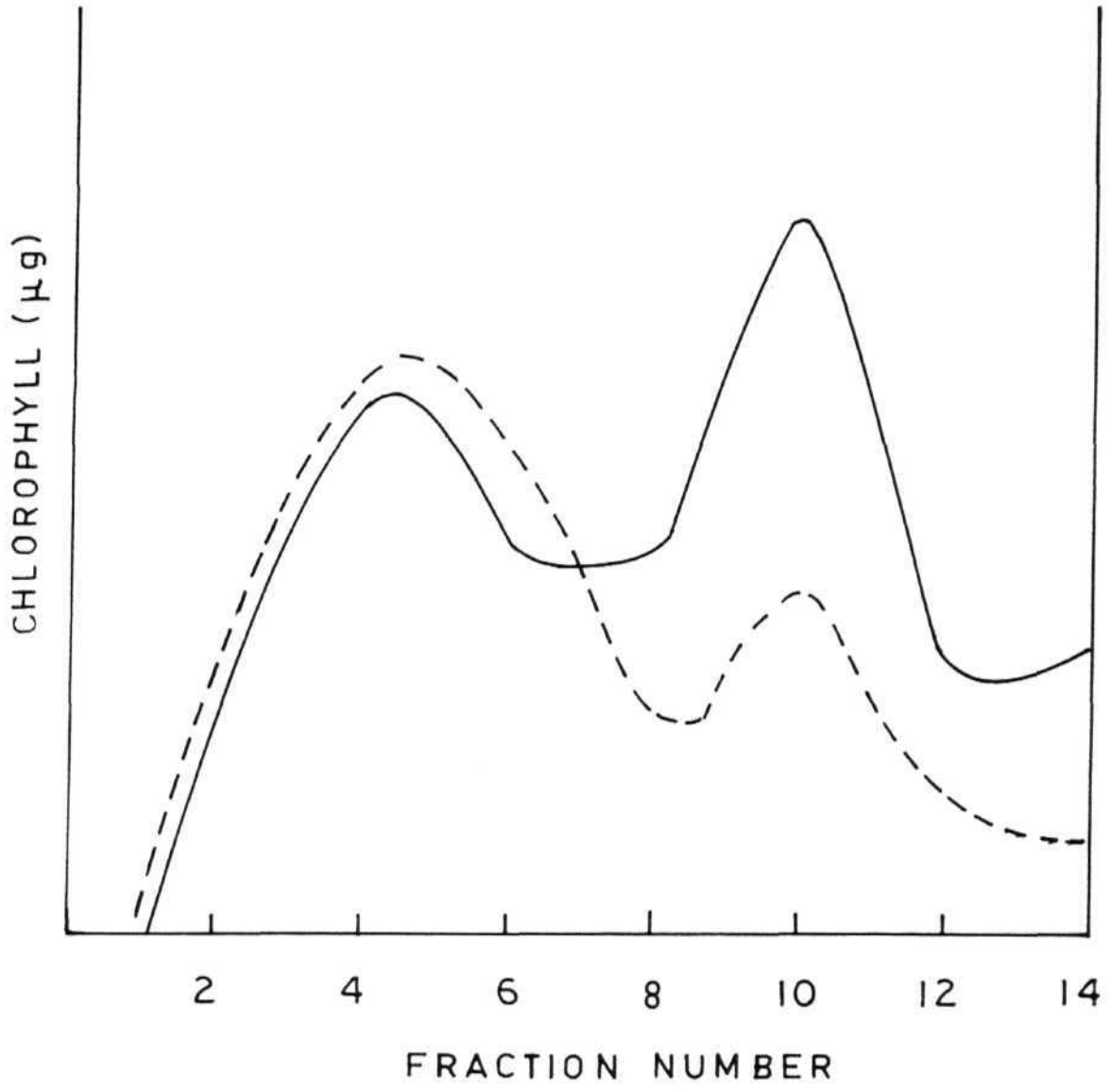


Figure 1.4: Isolation of PSII reaction center core: Elution profile of the anion exchange column.. $MgSO_4$ (0 200 mM) was used for washing the column.

——— Chlorophyll concentration plotted against fraction number.

- - - - Silicomolybdate reduction with DPC as electron donor plotted against fraction number.

Figure 1.5: **Fluorescence emission spectra of PS IT complex from Amaranthus hypochondriacus, L. at 77° K.**

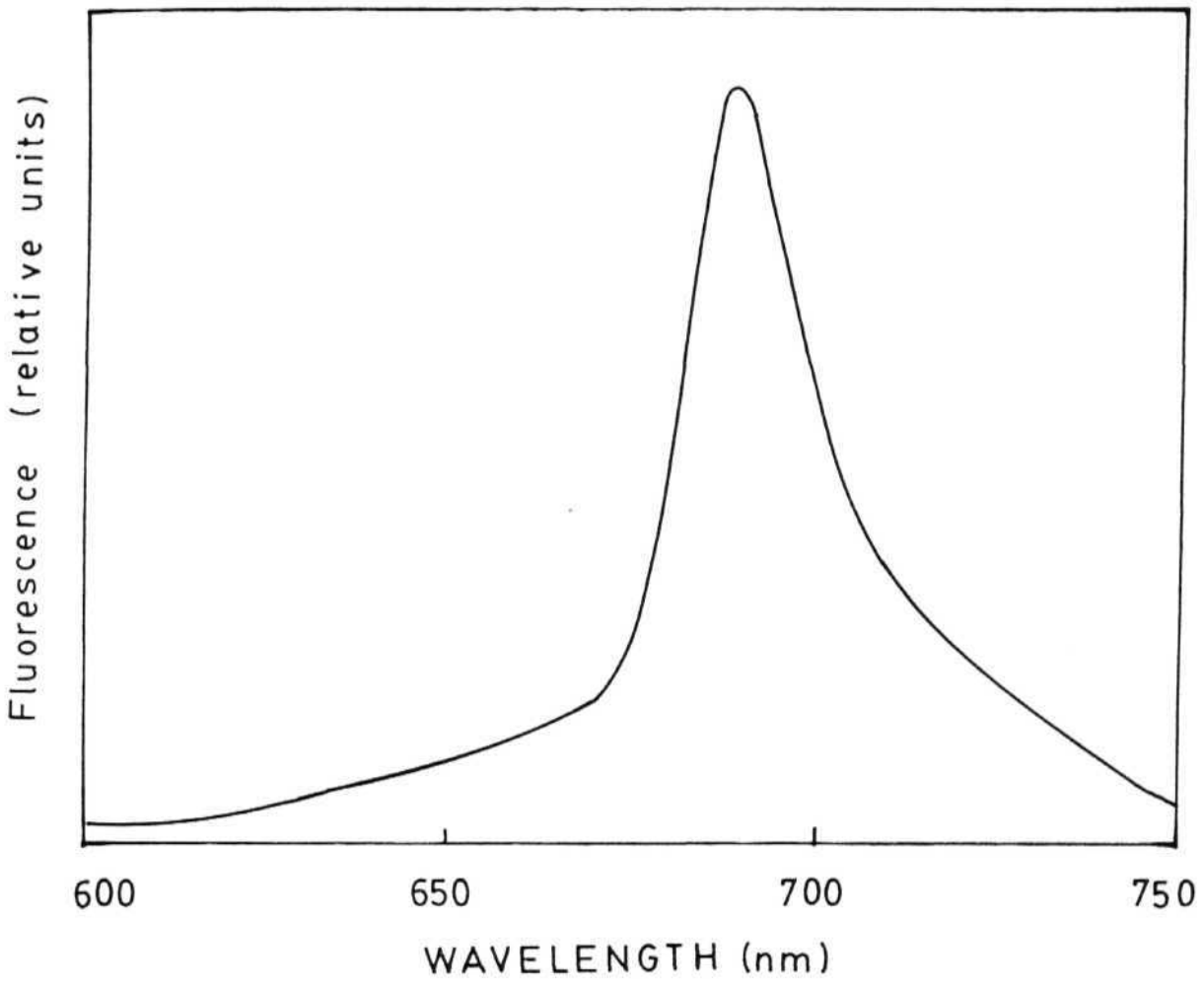


figure 1.6. EPR spectra of PS II complex from Amaranthus hypochondriacus. L.

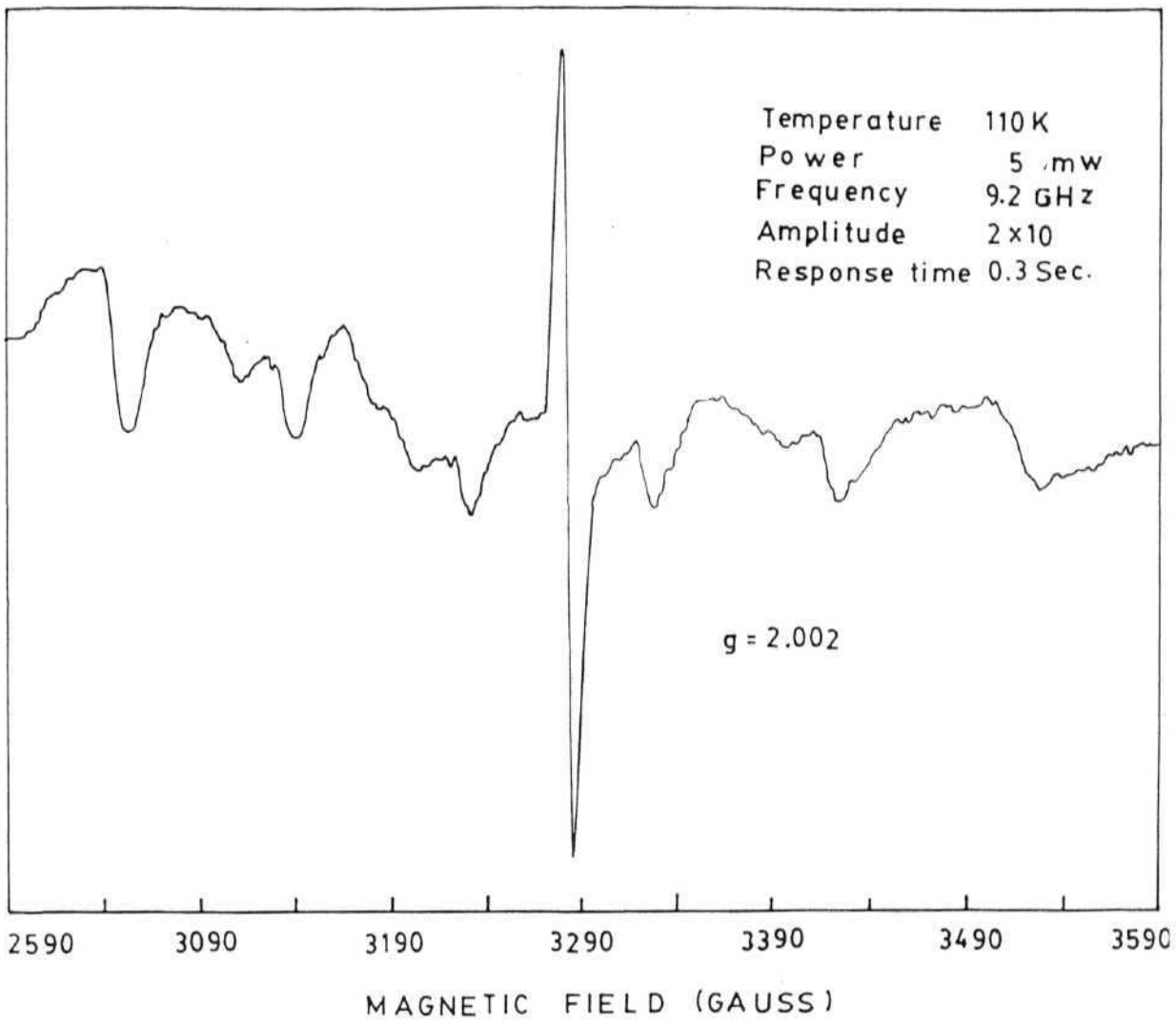


Figure 2.1a. Plants of *Amaranthus hypochondriacus*. L under various light regimes. H: Plants under normal sunlight ($2000\mu E\ m^{-2}s^{-1}$) L: plants under reduced irradiance ($650\mu E\ m^{-2}s^{-1}$). L_2: plants grown under reduced irradiance (200/iE $m^{-2}s^{-1}$).

Figure 2.1b. *Amamnthus hypochondriacus.* L plants acclimated to reduced irradiance. $H \rightarrow L_1$: plants grown under normal irradiance ($2000\mu E\ m^{-2}s^{-1}$) were transferred to reduced irradiance ($650\mu E\ m^{-2}s^{-1}$). $L_1 \rightarrow H$: plants grown under reduced irradiance (650/iE $m^{-2}s^{-1}$) were transferred to normal light ($2000\mu E\ m^{-2}s^{-1}$).


H
L I
L II

Figure 2.2a. Plants of *Eleusine coracana* under various light regimes. H: Plants grown under normal sunlight (2000μE $m^{-2}s^{-1}$) L_1: plants grown under reduced irradiance (650μE $m^{-2}s^{-1}$). L_2: Plants grown under reduced irradiance (200 μE $m^{-2}s^{-1}$).

Figure 2.2b Plants of *Eleusine coracana* under various light regimes.

$H \rightarrow L_1$ Plants gorwn under normal sunlight (2000 μE $m^{-2}s^{-1}$) on transfer to reduced irradiance (650 μE $m^{-2}s^{-1}$).

$H \rightarrow L_2$ Plants gorwn under normal sunlight (2000 μE $m^{-2}s^{-1}$) on transfer to reduced irradiance (200 μE $m^{-2}s^{-1}$).

$L_1 \rightarrow H$ Plants gorwn under reduced light (650 μE $m^{-2}s^{-1}$) on transfer to high irradiance (2000 μE $m^{-2}s^{-1}$).

$L_2 \rightarrow H$ Plants gorwn under reduced light (200 μE $m^{-2}s^{-1}$) on transfer to high irradiance (2000 μE $m^{-2}s^{-1}$).

Figure 2.3a Plants of *Gomphrena globasa* under various light regimes.

H: Plants grown under normal sun light (2000μE $m^{-2}s^{-1}$) L_1: Plants grown under reduced light (650μE $m^{-2}s^{-1}$) L_2: Plants grown under reduced light (200μE $m^{-2}s^{-1}$)

Figure 2.3b Plants of *Gomphrena globasa* under various light regimes.

$H \rightarrow L_1$ Plants gorwn under normal sunlight (2000 μE $m^{-2}s^{-1}$) on transfer to reduced irradiance (650 μE $m^{-2}s^{-1}$).

$H \rightarrow L_2$ Plants gorwn under normal sunlight (2000 μE $m^{-2}s^{-1}$) on transfer to reduced irradiance (200 μE $m^{-2}s^{-1}$).

$L_1 \rightarrow H$ Plants gorwn under reduced light (650 μE $m^{-2}s^{-1}$) on transfer to high irradiance (2000 μE $m^{-2}s^{-1}$).

$L_2 \rightarrow H$ Plants gorwn under reduced light (200 μE $m^{-2}s^{-1}$) on transfer to high irradiance (2000 μE $m^{-2}s^{-1}$).

Figure 2.4a. Growth light intensity effect on chlorophyll a/b ratio in *Amaranthus hypochondriacus* L.

0—0 Control plants grown at normal irradiance of 2000μE $m^{-2}s^{-1}$. [H].

▲—▲ Control plants grown at reduced irradiance of 650μE $m^{-2}s^{-1}$ [L_1].

●—● plants grown under normal irradiance (2000μE $m^{-2}s^{-1}$) were transferred to reduced irradiance (650μE $m^{-2}s^{-1}$) ($H \rightarrow L_1$).

△—△ Plants grown under low irradiance (650μE $m^{-2}s^{-1}$) were transferred to high irradiance (2000 μE $m^{-2}s^{-1}$) ($L_1 \rightarrow H$).

Figure 2.4b Effect of growth light intensity on chlorophyll a/b ratio in *Amaranthus hypochondriacus*. L.

0—0 Control plants grown at normal irradiance of 2000 μE $m^{-2}s^{-1}$ (H)

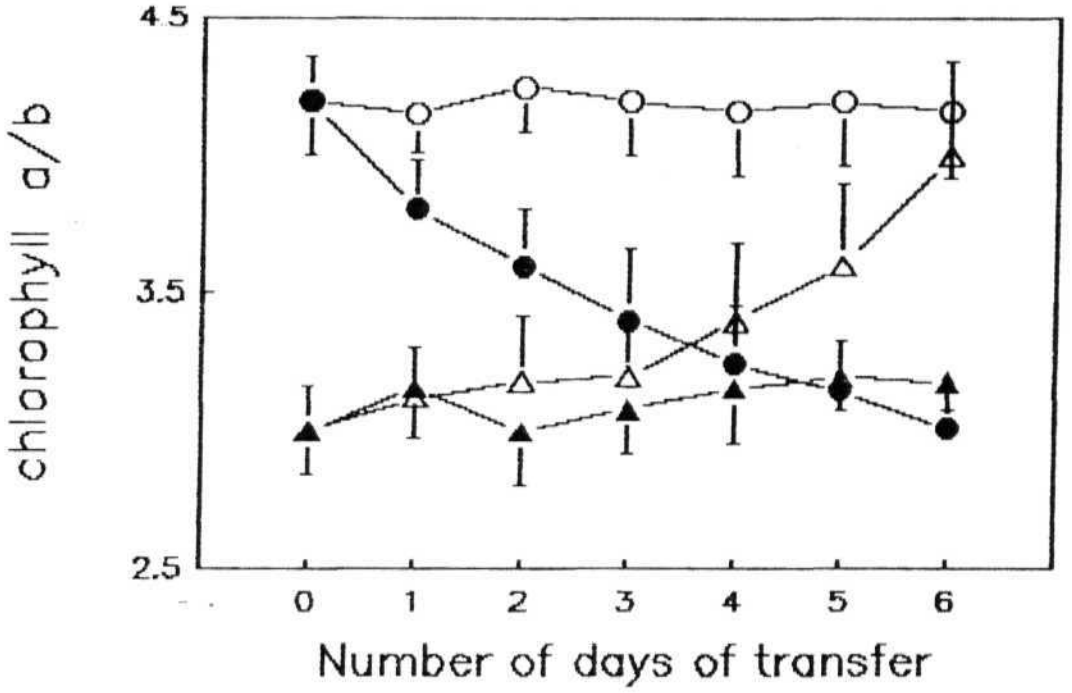
▼—▼ Control plants grown at reduced irradiance of 200 μE$m^{-2}s^{-1}$). (L_2).

□—□ Plants grown under high irradiance (2000 μE$m^{-2}s^{-1}$) were transferred to reduced irradiance of (200 μE$m^{-2}s^{-1}$). ($H \rightarrow L_2$). ■—■ Plants grown at reduced irradiance (200 μE $m^{-2}s^{-1}$ were transferred to high irradiance (2000μE $m^{-2}s^{-1}$) ($L_2 \rightarrow H$)

The results are average of ten independent values.

NOTE : Abbreviations of legends of plants grown at various light intensities are used hereafter. For full form of legends this page could be refered.

2.4a


4.5
3.5
2.5
chlaraphyll a/b
0
1
2
3
4
5
6
Number of days of transfer

2.4b

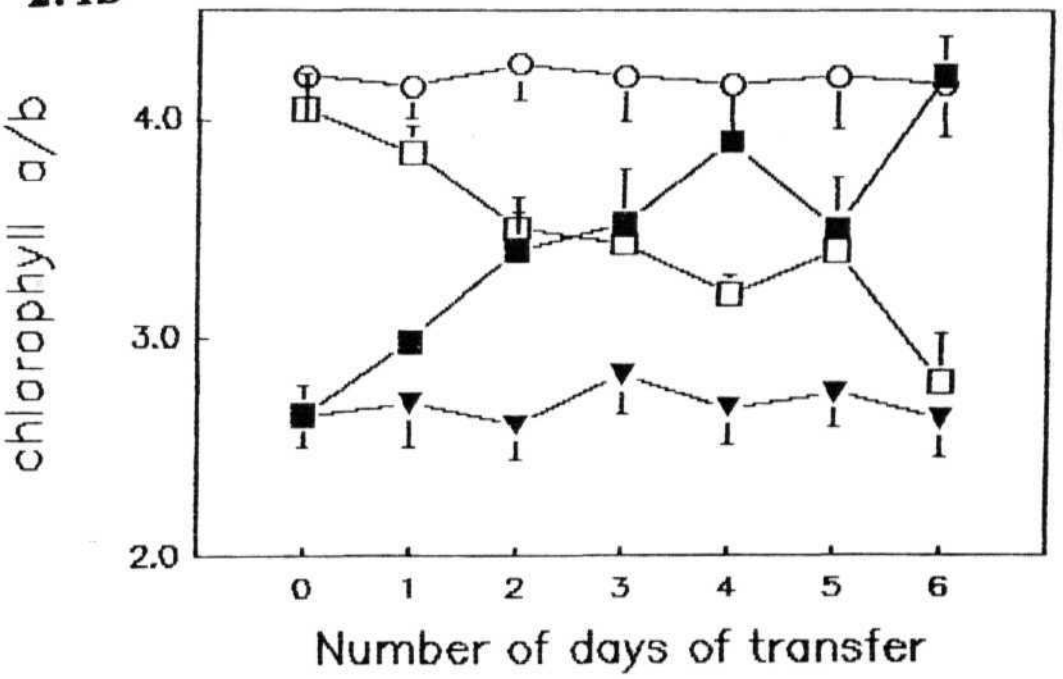

4.0
3.0
2.0
chlaraphyll a/b
0
1
2
3
4
5
6
Number of days of transfer

Figure 2.5a Effect of growth light intensity on chlorophyll *a/b* ratio in *Eleusint coracana.*

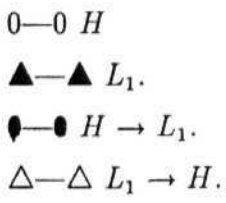
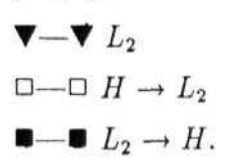
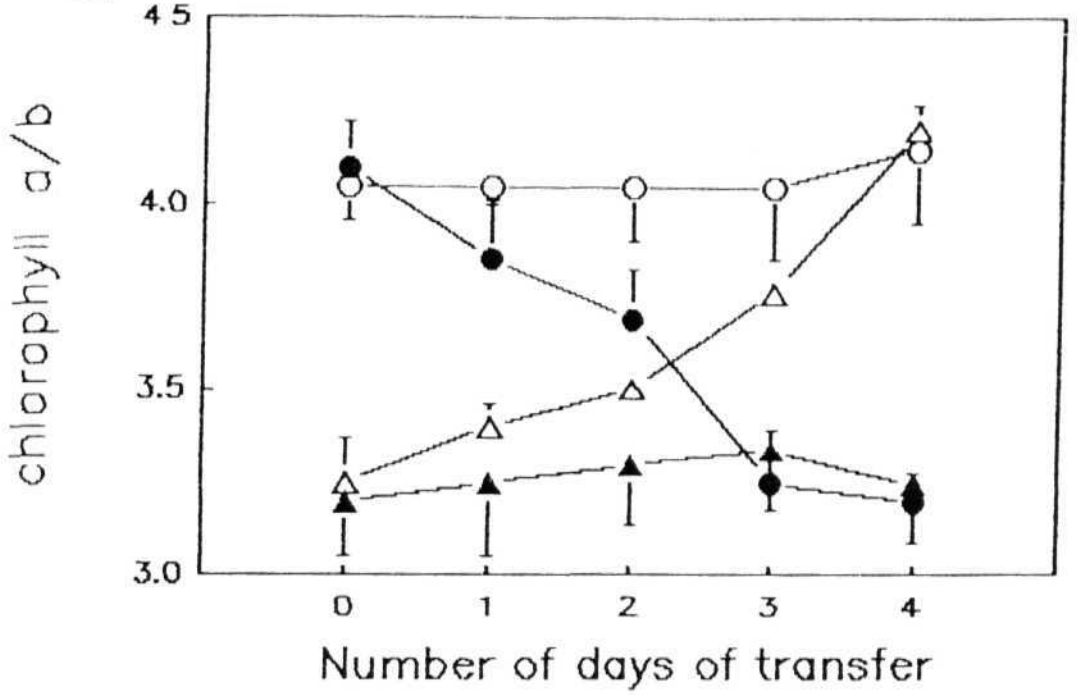


Figure 2.5b Effect of growth light intensity on chlorophyll *a/b* ratio in *Eleusint coracana.*



2.5a


chlorophyll a/b
4.5
4.0
3.5
3.0
0
1
2
3
4
Number of days of transfer

2.5b

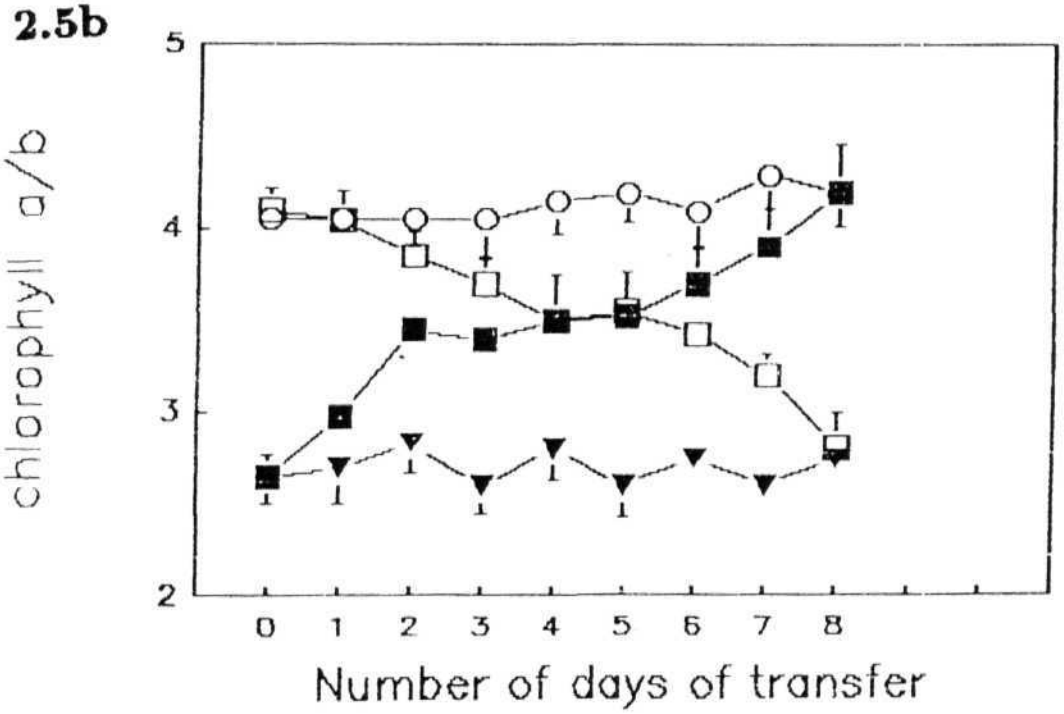

chlorophyll a/b
5
4
3
2
0
1
2
3
4
5
6
7
8
Number of days of transfer

Figure 2.6a Effect of growth light intensity on chlorophyll *a/b* ratio in *Gomphrena globosa.*

0—0 H

▲—▲ L_1

⬮—⬮ $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

Figure 2.6b Effect of growth light intensity on chlorophyll *a/b* ratio in *Gomphrena globosa.*

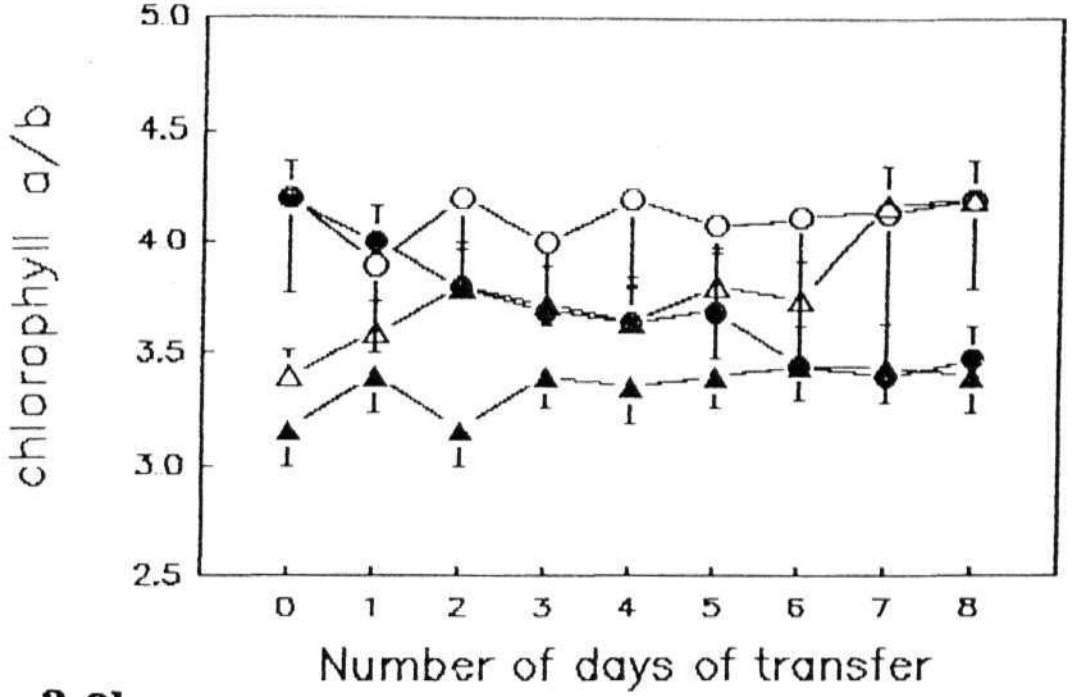
0—0 H

▼—▼ L_2

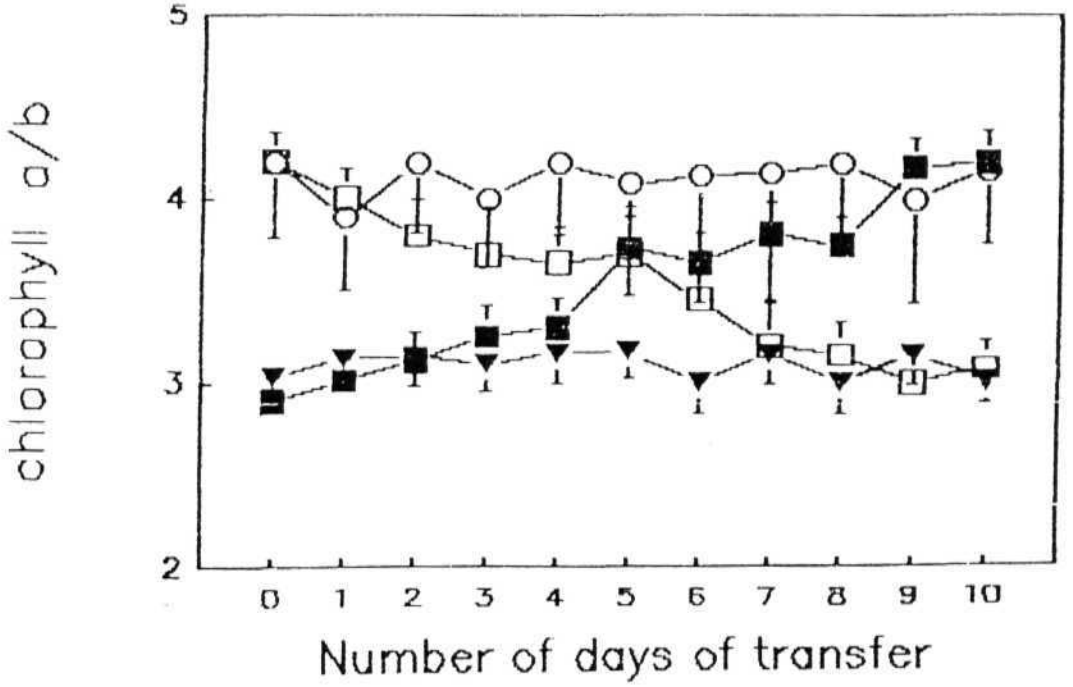
□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

2.6a


chloraphyll a/b
5.0
4.5
4.0
3.5
3.0
2.5
0 1 2 3 4 5 6 7 8
Number of days of transfer

2.6b


chloraphyll a/b
5
4
3
2
0 1 2 3 4 5 6 7 8 9 10
Number of days of transfer

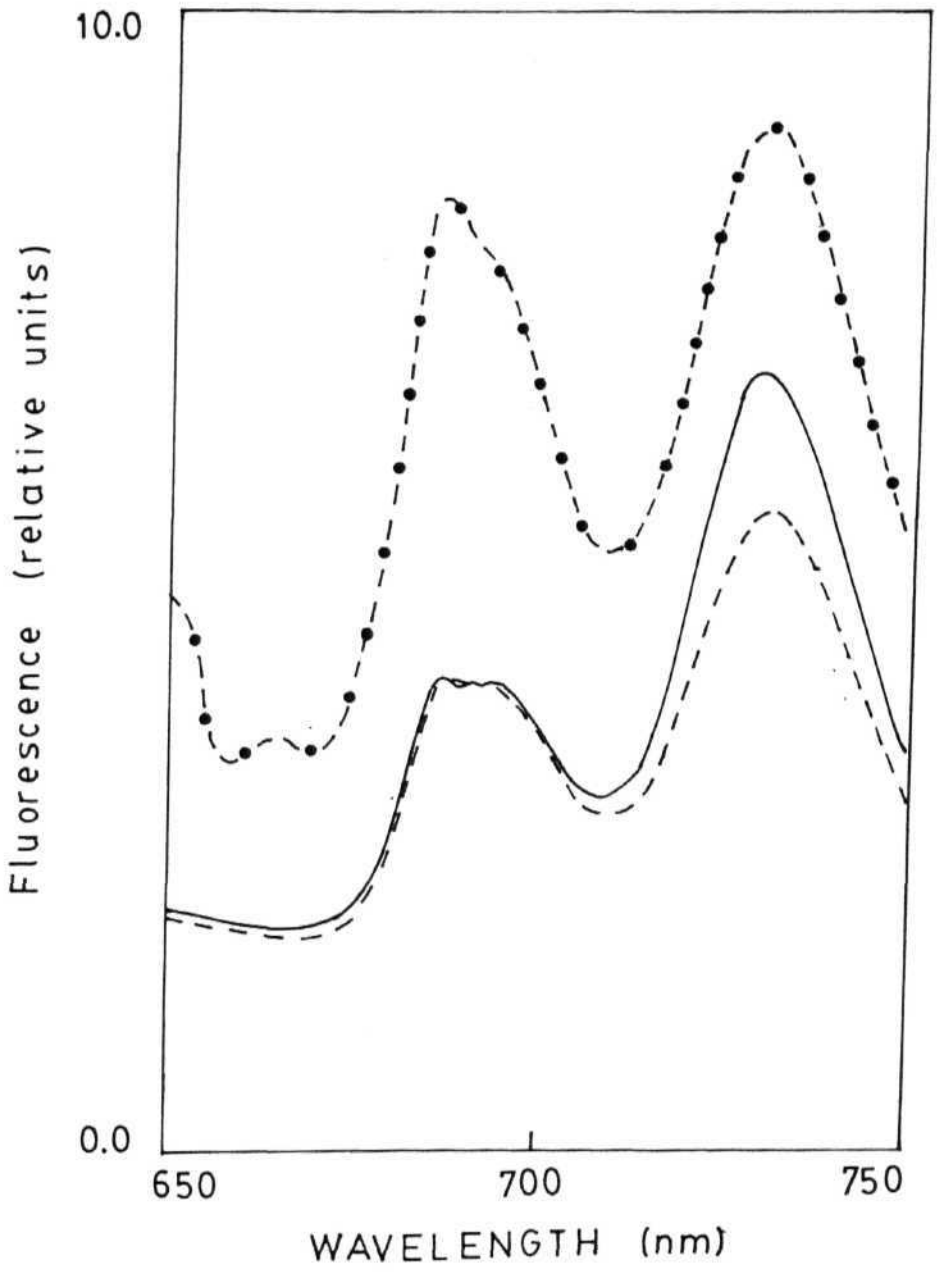
irradiance (Figures 2.4a, 2.4b, 2.5a, 2.5b, 2.6a and 2.6b). *ti* $\rightarrow L_1$ and $H \rightarrow L_2$ plants of *Amaranthus*, *Eleusine* and *Gomphrena* showed reduced chlorophyll *a/b* ratio while increased ratio was observed in $L_1 \rightarrow H$ and $L_2 \rightarrow H$ plants (Figures 2.4a, 2.4b, 2.5a, 2.5b, 2.6a and 2.6b). The time taken for $H \rightarrow L_1$ and $L_1 \rightarrow H$ plants to show the changes in chlorophyll *a/b* ratio in *Amaranthus*, *Eleusine* and *Gomphrena* were six, four and eight days respectively whereas $H \rightarrow L_2$ and $L_2 \rightarrow H$ plants required six, eight and ten days respectively. An initial time lag was observed for *Gomphrena*.

Fluorescence

Fluorescence emission spectra at 77°K of *Amaranthus* thylakoid membranes exhibited peaks at 690 nm and 735 nm (Figure 2.7). The thylakoid membranes of L_1 and L_2 plants showed increased F_{690} peak compared to F_{735}. F_{690}/F_{735} ratio at 77°K increased by 94% and 180% in L_1 and L_2 *Amaranthus* plants respectively (Figure 2.7 and Table 2.2). The F_{690}/F_{735} ratio at 77°K increased by 72% and 136% in mesophyll thylakoids while 93% and 194% increase was observed in bundle sheath of L_1 and L_2 plants of *Amaranthus* (Table 2.2). F_{690}/F_{735} ratio at room temperature increased by 100% and 150% in mesophyll thylakoids whereas 84% and 188% increase was observed in bundle sheath of L_1 and L_2 *Amaranthus* plants respectively (Table 2.1). The increase in F_{690}/F_{735} ratio in response to changes in growth irradiance at both room and liquid nitrogen temperatures was higher in bundle sheath thylakoids compared to mesophyll (Tables 2.1 and 2.2).

The fluorescence emission spectra at 77°K of H, L_1 and L_2 plants of *Eleusine* and *Gomphrena* are given in figures 2.8 and 2.9. F_{690} peak increased in plants grown under reduced irradiances. The L_1 and L_2 plants of *Eleusine* exhibited 112% and 165% increase in F_{690}/F_{735} ratio at 77°K while 70% and 121% increase was observed in the similar plants

Figure 2.7: Fluorescence emission spectra in Amaranthus hypochondriacus. L. at 77°K.



——— High light grown (2000 $\mu E\ m^{-2}s^{-1}$)

- - - - - low light grown (650 $\mu E\ m^{-2}s^{-1}$)

—·—·— low light grown (200 $\mu E\ m^{-2}s^{-1}$)

Excitation wavelength: 490nm

Figure 2.8: Fluorescence emission spectra of thylakoid membranes in Eleusine coracana at 77°K. Excitation wavelength: 490 nm

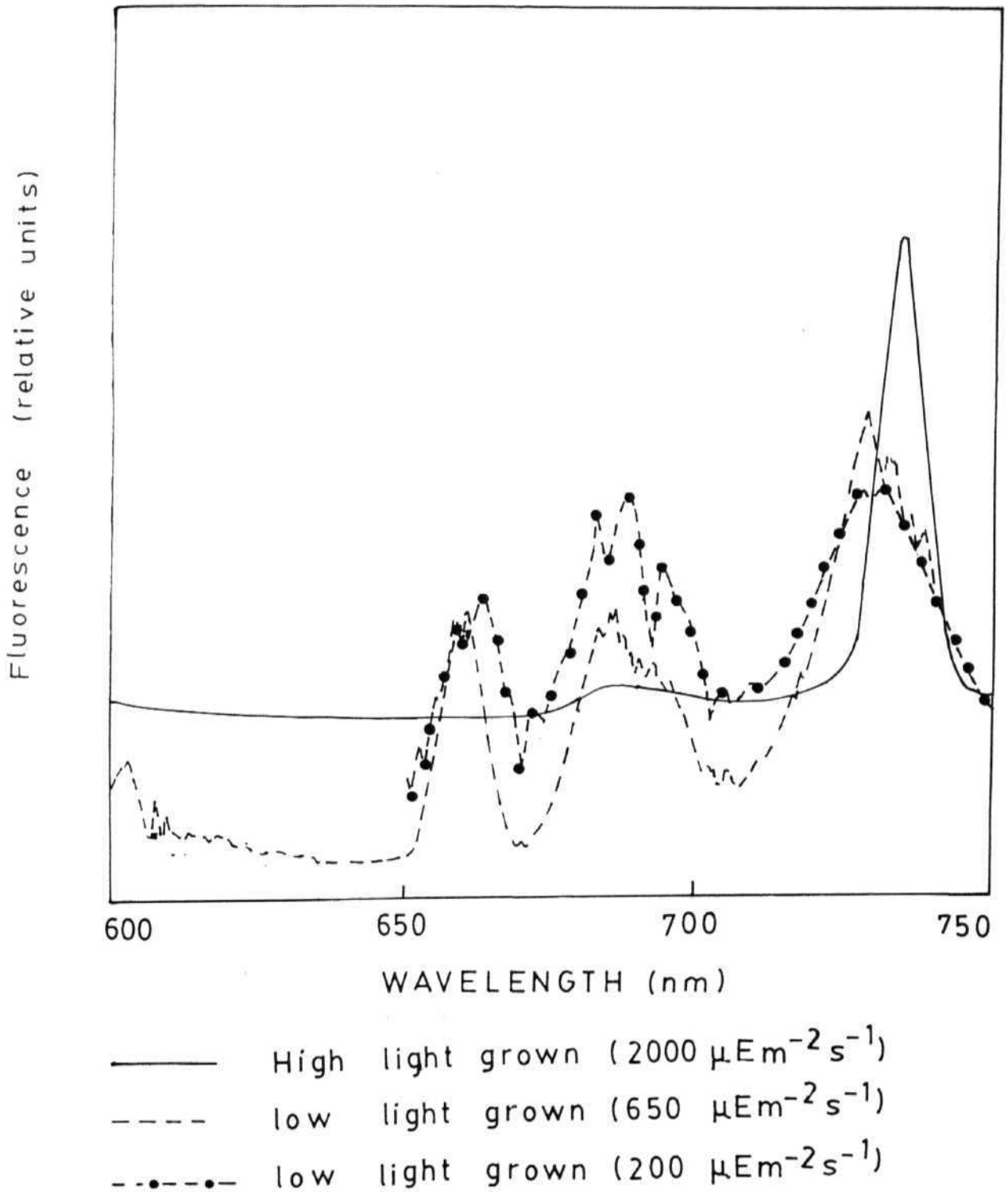


Figure 2.9: Fluorescence emission spectra of thylakoid membranes of Gomphrena globosa at 77°K.

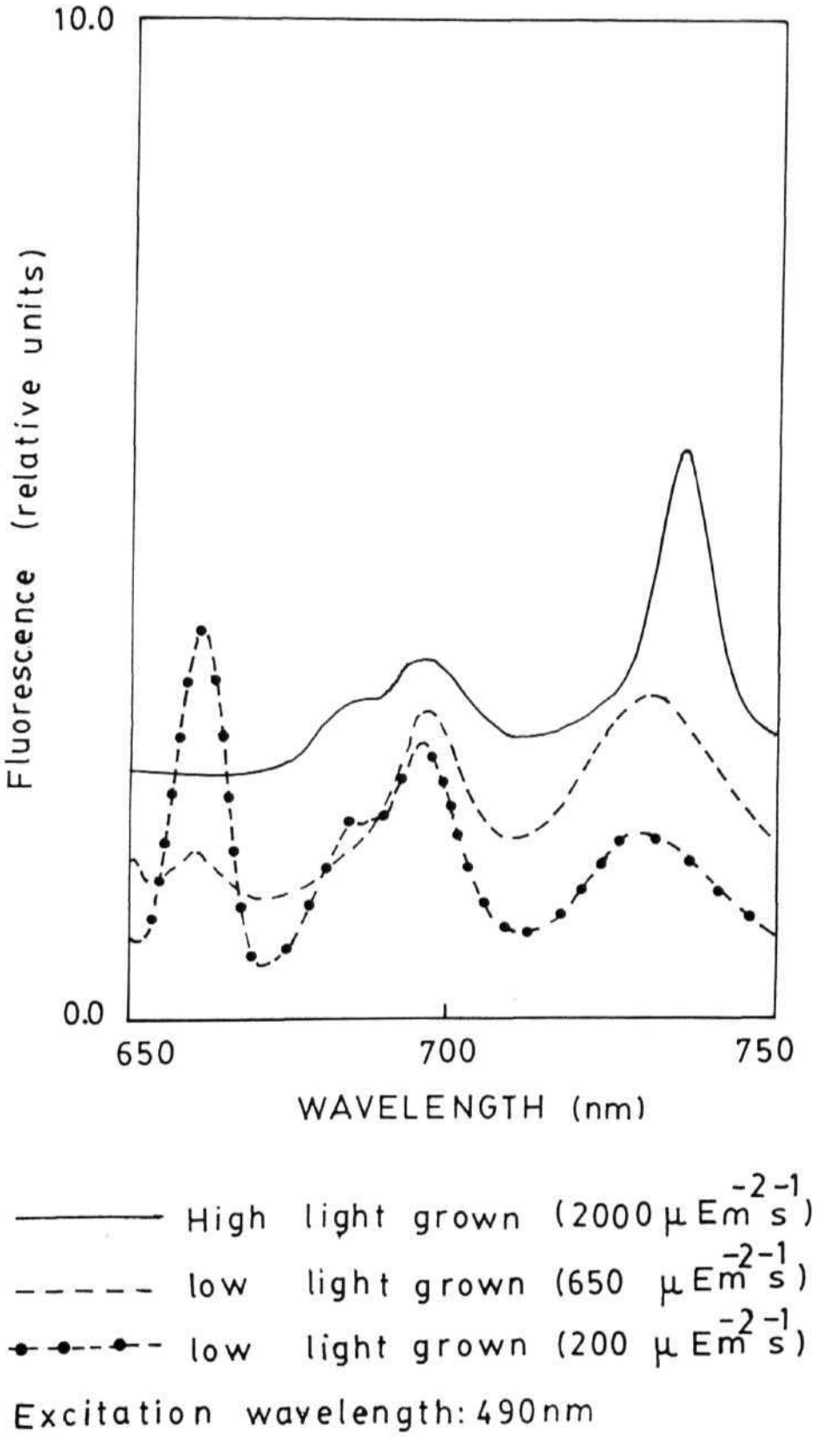


Table 2.1: Effect of low light intensity on F_{690}/F_{735} ratio at room temperature

Plant Species	F_{690}/F_{35} ratio at R.T.			% decrease of control on acclimation		% increase of control on acclimation	
	H	L_1	L_2	$L_1 \rightarrow H$	$L_2 \rightarrow H$	$H \rightarrow L_1$	$H \rightarrow L_2$
A. hypochondriacus							
mesophyll	0.52±0.18	1.04±0.14[a]	1.29±0.25[a]	48	59	100	148
bundle sheath	0.76±0.06	1.43±0.15[a]	2.19±0.14[a]	50	65	84	188
E. coracana	0.84±0.12	1.51±0.12[a]	2.10±0.23[a]	44	60	79	150
G. globosa	0.79±0.1	1.40±0.2[a]	1.76±0.13[a]	43	56	77	123

The values are average of 10 individual readings

H : Thylakoid membranes from high light (2000 $\mu E\ m^{-2}s^{-1}$) grown plants.

L_1: Thylakoid membranes from low light (650 $\mu E\ m^{-2}s^{-1}$) grown plants.

L_2: Thylakoid membranes from low light (200 $\mu E\ m^{-2}s^{-1}$) grown plants.

$L_1 \rightarrow H$: Thylakoid membranes from low light (650 $\mu E\ m^{-2}s^{-1}$) grown plants after acclimation to high irradiance (2000 $\mu E\ m^{-2}s^{-1}$).

$L_2 \rightarrow H$: Thylakoid membranes from low light (200 $\mu E\ m^{-2}s^{-1}$) grown plants acclimated to high irradiance (2000 $\mu E\ m^{-2}s^{-1}$).

$H \rightarrow L_1$: Thylakoid membranes from high irradiance (2000 $\mu E\ m^{-2}s^{-1}$) grown plants acclimated to reduced light (650 $\mu E\ m^{-2}s^{-1}$).

$H \rightarrow L_2$: Thylakoid membranes from high irradiance (2000 $\mu E\ m^{-2}s^{-1}$) grown plants acclimated to reduced light (200 $\mu E\ m^{-2}s^{-1}$).

a = $p < 0.001$

Table 2.2: Effect of low light intensity on F_{690}/F_{735} ratio at 77° K.

Plant Species	F_{690}/F_{35} 77°K			% decrease of control on acclimation		% increase of control on acclimation	
	H	L_1	L_2	$L_1 \rightarrow H$	$L_2 \rightarrow H$	$H \rightarrow L_1$	$H \rightarrow L_2$
A. hypochondriacus							
mesophyll	0.34±0.02	0.57±0.02[a]	0.78±0.04[a]	43	58	72.0	136
bundle sheath	0.35±0.04	0.68±0.04[a]	1.03±0.10[a]	48	66	93.0	194
E. coracana	0.40±0.06	0.85±0.04[a]	1.08±0.02[a]	52	67	112.5	165
G. globosa	0.51±0.70	0.87±0.04[a]	1.13±0.02[a]	41	54	70.0	121

The values are average of 10 individual readings

H : Thylakoid membranes from high light (2000 μE $m^{-2}s^{-1}$ grown plants.

L_1: Thylakoid membranes from low light (650 μE $m^{-2}s^{-1}$) grown plants.

L_2: Thylakoid membranes from low light (200 μE $m^{-2}s^{-1}$) grown plants.

$L_1 \rightarrow H$: Thylakoid membranes from low light (650 μE $m^{-2}s^{-1}$) grown plants after acclimation to high irradiance (2000 μE $m^{-2}s^{-1}$).

$L_2 \rightarrow H$: Thylakoid membranes from low light (200 μE $m^{-2}s^{-1}$) grown plants acclimated to high irradiance (2000 μE $m^{-2}s^{-1}$).

$H \rightarrow L_1$: Thylakoid membranes from high irradiance (2000μE $m^{-2}s^{-1}$) grown plants acclimated to reduced light (650 μE $m^{-2}s^{-1}$).

$H \rightarrow L_2$: Thylakoid membranes from high irradiance (2000 μE $m^{-2}s^{-1}$) grown plants acclimated to reduced light (200 μE $m^{-2}s^{-1}$).

a = $p < 0.001$

of *Gomphrena* respectively (Table 2.2). In L_1 and L_2 plants of *Eleusine*, F_{690}/F_{735} ratio at room temperature increased by 79% and 150% respectively while similar plants in *Gomphrena* showed 77% and 123% increase in the ratio (Table 2.1).

A typical *in vivo* fluorescence induction curve at 77^{o}K for dark adapted *Amaranihus* leaf is given in figure 2.10. In L_1 and L_2 plants of *Amaranthus* F_o (ground level fluorescence) increased and F_m (fluorescence maxima) decreased (Figure 2.10). F_v/F_m ratio decreased by 62% and 66% at room temperature in $L\backslash$ and L_2 plants of *Amaranthus* but in similar plants the decrease in the ratio was 19% and 24% at 77^{o}K.

F_o increased and F_m decreased in L_1 and L_2 plants of *Eleusinc* and *Gomphrena* (Figures 2.11 and 2.12). L_1 and L_2 *Eleusine* plants showed 54% and 65% reduction in F_v/F_m at room temperature whereas 11% and 25% decrease in the ratio was observed at 77^{o}K (Tables 2.3 and 2.4). F_v/F_m at room temperature decreased by 53% and 62% in L_1 and L_2 plants of *Gomphrena* while only 10% and 20% reduction in the ratio was observed at 77^{o}K, respectively (Tables 2.3 and 2.4).

In $L_1 \rightarrow$ // and $L_2 \rightarrow H$ plants of *Amaranthus,* 43% and 58% reduction was observed in F_{690}/F_{735} ratio at 77^{o}K in mesophyll thylakoids whereas 48% and 66% decrease was exhibited in bundle sheath thylakoid membranes in a span of six days(Figures 2.13a, 2.13b, 2.14a and 2.14b; Table 2.2). The F_{690}/F_{735} ratio at room temperature decreased by 48% and 59% in mesophyll whereas 59% and 65% decrease was observed in bundle sheath of $L_1 \rightarrow H$ and $L_2 \rightarrow H$ *Amaranthus* plants (Figures 2.15a, 2.15b, 2.16a and 2.16b). F_{690}/F_{735} ratio at 77^{o}K increased by 72% and 136% in $H \rightarrow L_1$ and $H \rightarrow L_2$ mesophyll thylakoids whereas 93% and 194% increase was observed in bundle sheath respectively (Figures 2.13a, 2.13b, 2.14a and 2.14b). $H \rightarrow L_1$ and $H \rightarrow L_2$ *Amaranthus*

Figure 2.10: Fluorescence induction kinetics at 77°K in Amaranthus hypochondriacus L.

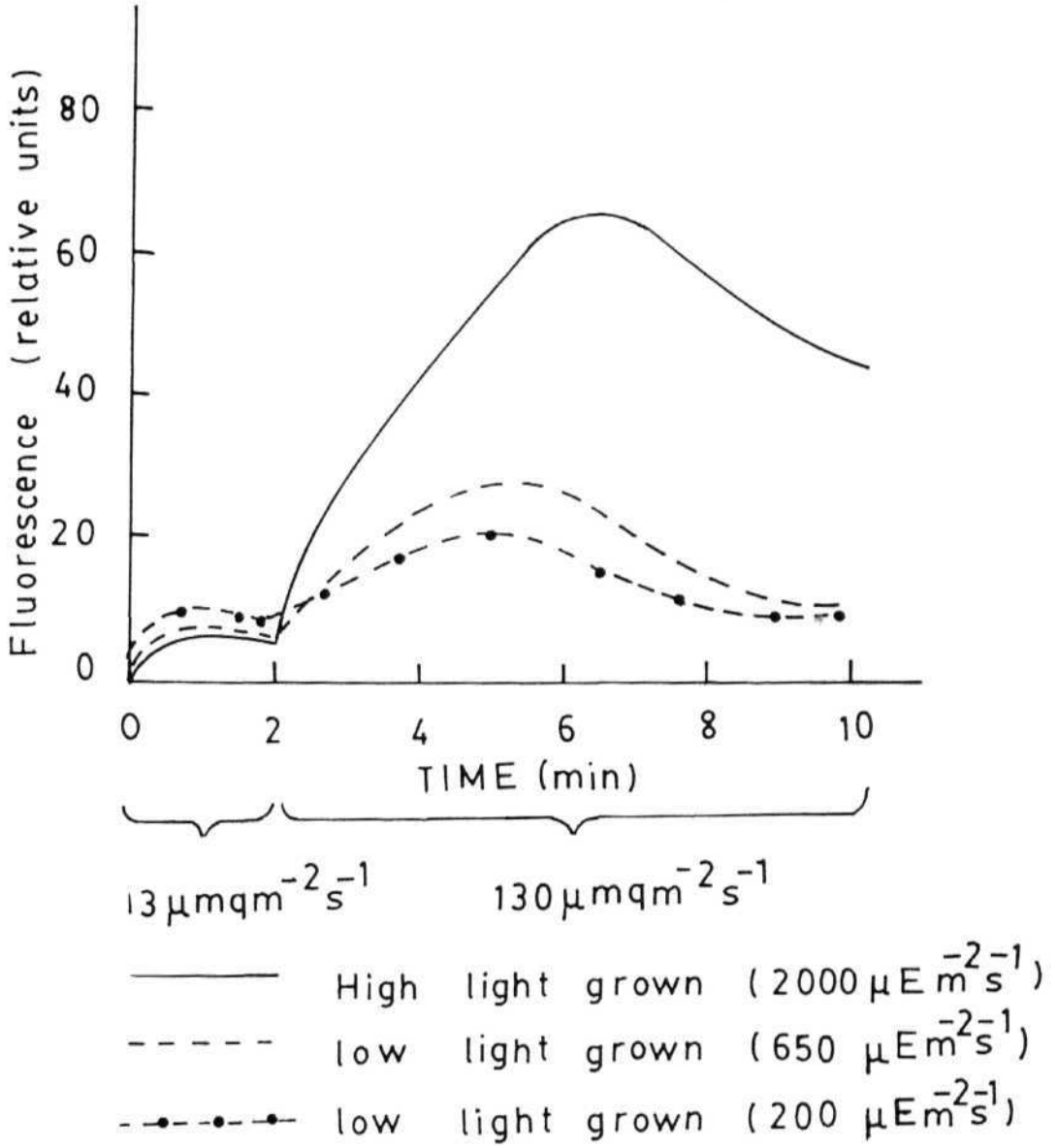


Figure 2.11: Fluorescence inductfon kinetics at 77° K in Eleusine coracana

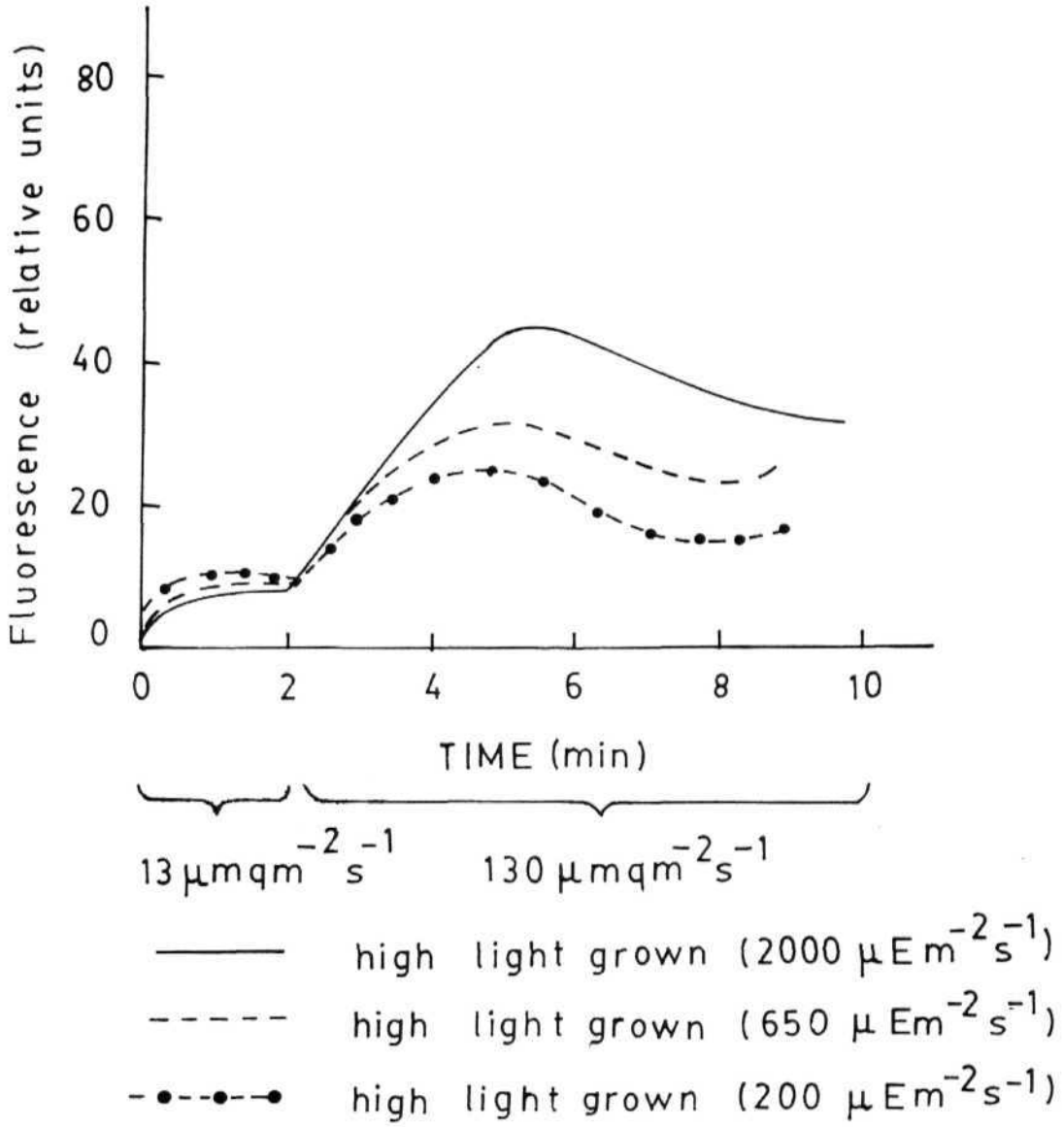


Figure.2.12: Fluorescence induction kinetics at 77° K in Gomphrena globosa.

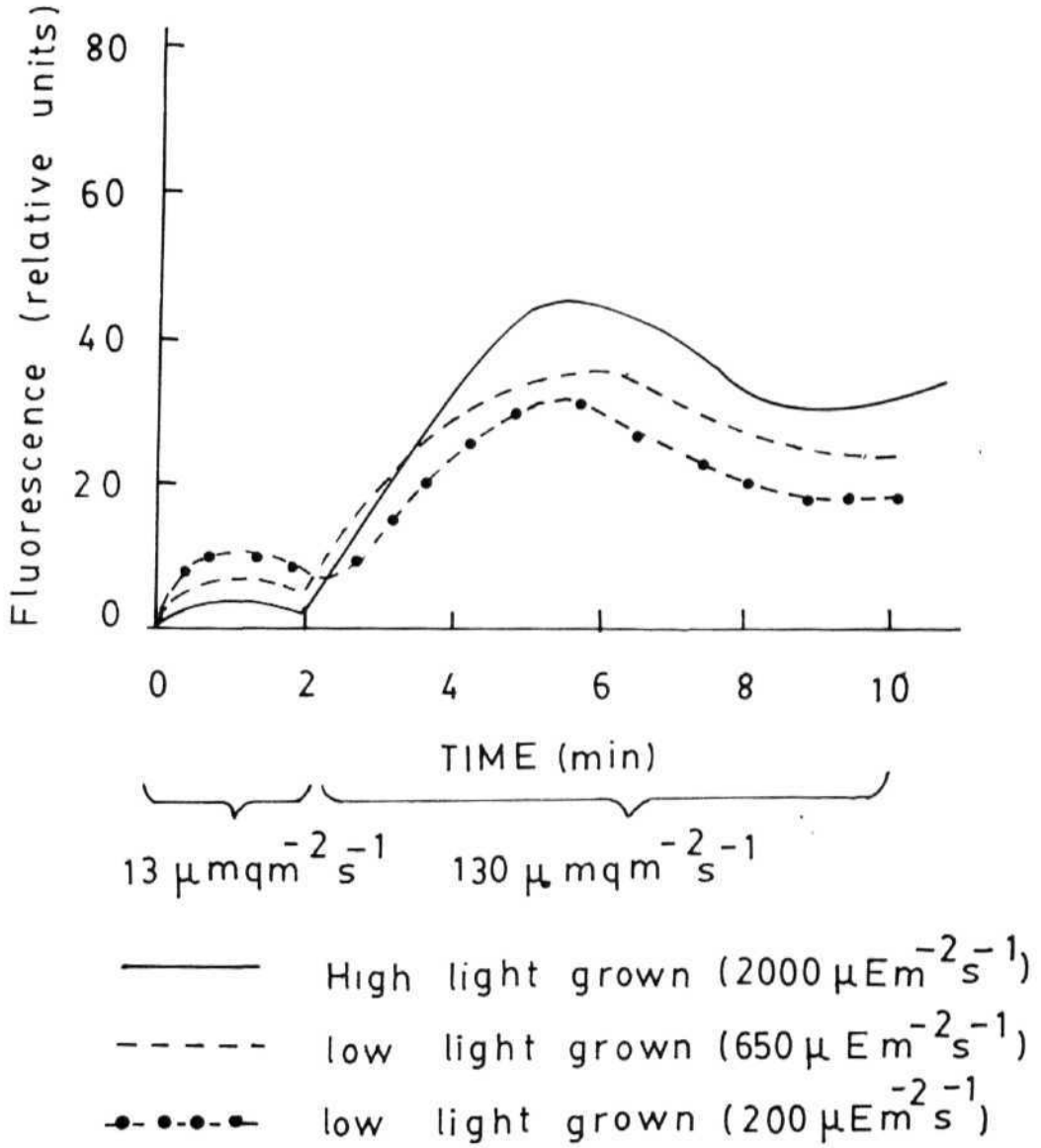


Table 2.3: Effect of light intensity on F_v/F_m ratio at room temperature

Plant Species	F_v/F_m			% decrease of control on acclimation		% increase of control on acc limation	
	H	L_1	L_2	$L_1 \rightarrow H$	$L_2 \rightarrow H$	$H \rightarrow L_1$	$H \rightarrow L_2$
A. hypochondriacus	0.57±0.06	0.24±0.04[a]	0.19±0.02[a]	62	66	125	200
E. coracana	0.51±0.09	0.23±0.04[b]	0.18±0.02[b]	54	65	121	183
G. globosa	0.57±0.06	0.23±0.04[a]	0.21±0.06[a]	53	62	115	164

The values are average of 10 individual readings

H : Leaves from high irradiance (2000 $\mu E\ m^{-2}s^{-1}$) plants.

L_1: Leaves from low irradiance (650 $\mu E\ m^{-2}s^{-1}$) plants.

L_2: Leaves from low irradiance (200 $\mu E\ m^{-2}s^{-1}$) plants.

$H \rightarrow L_1$: Leaves from high irradiance grown plants after acclimation to reduced irradiance (650 $\mu E\ m^{-2}s^{-1}$)

$H \rightarrow L_2$: Leaves from high irradiance grown plants after acclimation to reduced irradiance (200 $\mu E\ m^{-2}s^{-1}$)

$L_1 \rightarrow H$: Leaves from low irradiance (650 $\mu E\ m^{-2}s^{-1}$) grown plants after acclimation to high irradiance.

$L_2 \rightarrow H$: Leaves from low irradiance (200 $\mu E\ m^{-2}s^{-1}$) grown plants after acclimation to high irradiance.

a = $p < 0.001$ b = $p < 0.005$

Table 2.4: Effect of low light intensity on F_v/F_m ratio at 77°K

Plant Species	F_v/F_m at 77°K			% decrease of control on acclimation		% increase of control on acclimation	
	H	L_1	L_2	$H \rightarrow L_1$	$H \rightarrow L_2$	$L_1 \rightarrow H$	$L_2 \rightarrow H$
A. hypochondriacus	0.84±0.06	0.68±0.03[a]	0.62±0.03[a]	19	24	23	35
E. coracana	0.84±0.06	0.74±0.05[c]	0.63±0.06[a]	11	25	13	33
G. globosa	0.86±0.04	0.77±0.04[b]	0.69±0.04[a]	10	20	12	24

H : Leaves from high irradiance (2000 $\mu E\ m^{-2}s^{-1}$) plants.

L_1: Leaves from irradiance (650 $\mu E\ m^{-2}s^{-1}$) plants.

L_2: Leaves from low irradiance (200 $\mu E\ m^{-2}s^{-1}$) plants.

$H \rightarrow L_1$: Leaves from high irraqdiance grown plants after acclimation to reduced irradiance (650 $\mu E\ m^{-2}s^{-1}$)

$H \rightarrow L_2$: Leaves from high irradiance grown plants after acclimation to reduced irradiance (200 $\mu E\ m^{-2}s^{-1}$)

$L_1 \rightarrow H$: Leaves from low irradiance (650 $\mu E\ m^{-2}s^{-1}$) grown plants after acclimation to high irradiance.

$L_2 \rightarrow H$: Leaves from low irradiance (200 $\mu E\ m^{-2}s^{-1}$) grown plants after acclimation to high irradiance.

a = $p < 0.001$ b = $p < 0.005$ c = $p < 0.02$

Figure 2.13a Effect of light intensity on F_{690}/F_{735} ratio at 77°K in mesophyll thylakoid membranes of *Amaranthus hypochondriacus* L.

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

Figure 2.13b Effect of light intensity on F_{690}/F_{735} ratio at 77°K in mesophyll thylakoid membranes of *Amaranthus hypochondriacus* L.

0—0 H

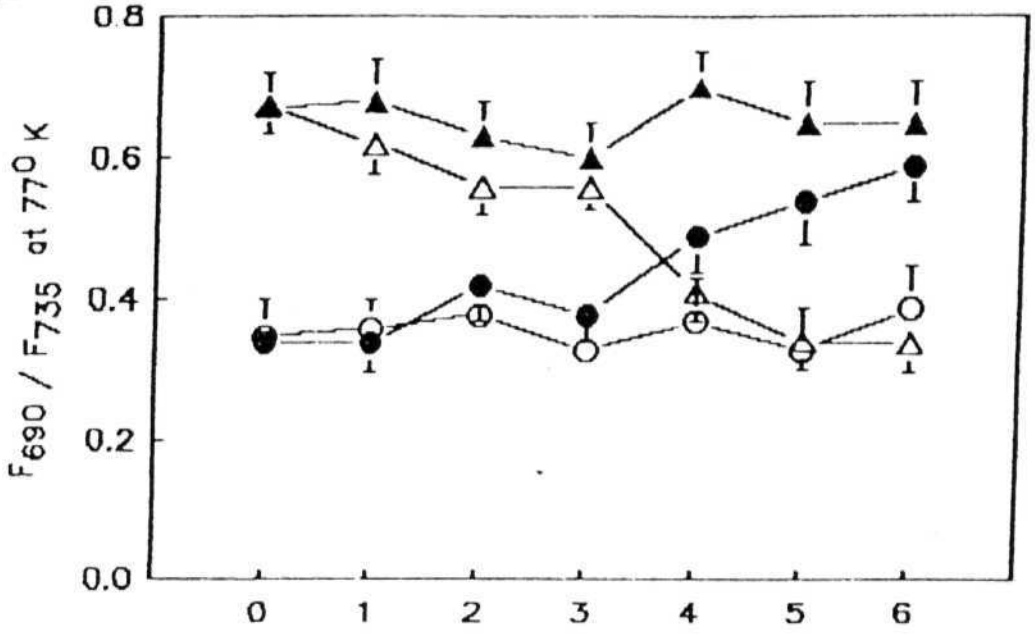
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three individual readings.

2.13a


0.8
0.6
0.4
0.2
0.0
F_{690} / F_{735} at 77^{0} K
0
1
2
3
4
5
6
Number of days of transfer

2.13b

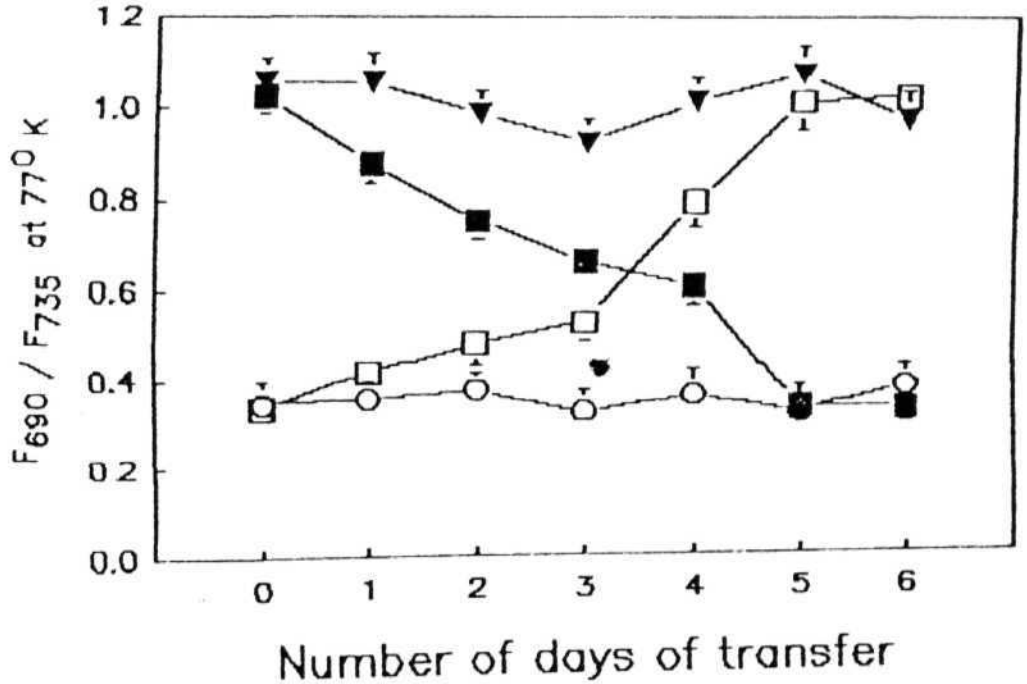

1.2
1.0
0.8
0.6
0.4
0.2
0.0
F_{690} / F_{735} at 77^{0} K
0
1
2
3
4
5
6
Number of days of transfer

Figure 2.14a Effect of light intensity variations on F_{690}/F_{735} ratio at 77°K in bundle sheath thylakoid membranes of *Amaranthus.*

0—0 H

▲—▲ L_1

⬮—⬮ $H \rightarrow L_1$

△—△ $L_2 \rightarrow H$.

Figure 2.14b Effect of light intensity variations on F_{690}/F_{735} ratio at 77°K in bundle sheath thylakoid membranes of *Amaranthus*.

0—0 H

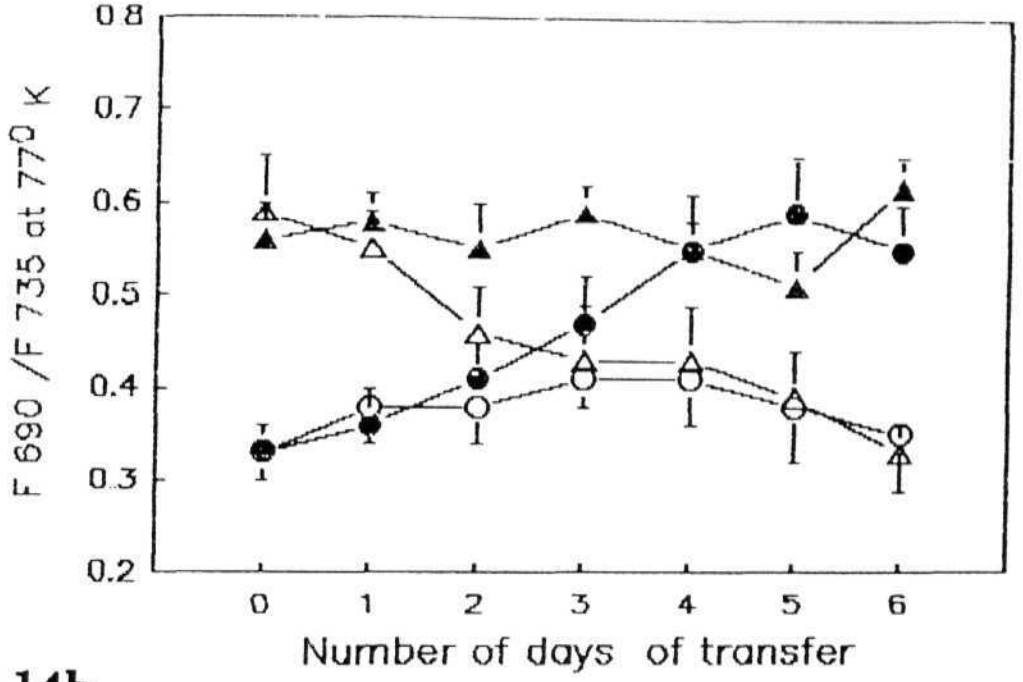
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three individual experiments.

2.14a


0.8
0.7
0.6
0.5
0.4
0.3
0.2
F 690 /F 735 at 77° K
0
1
2
3
4
5
6
Number of days of transfer

2.14b

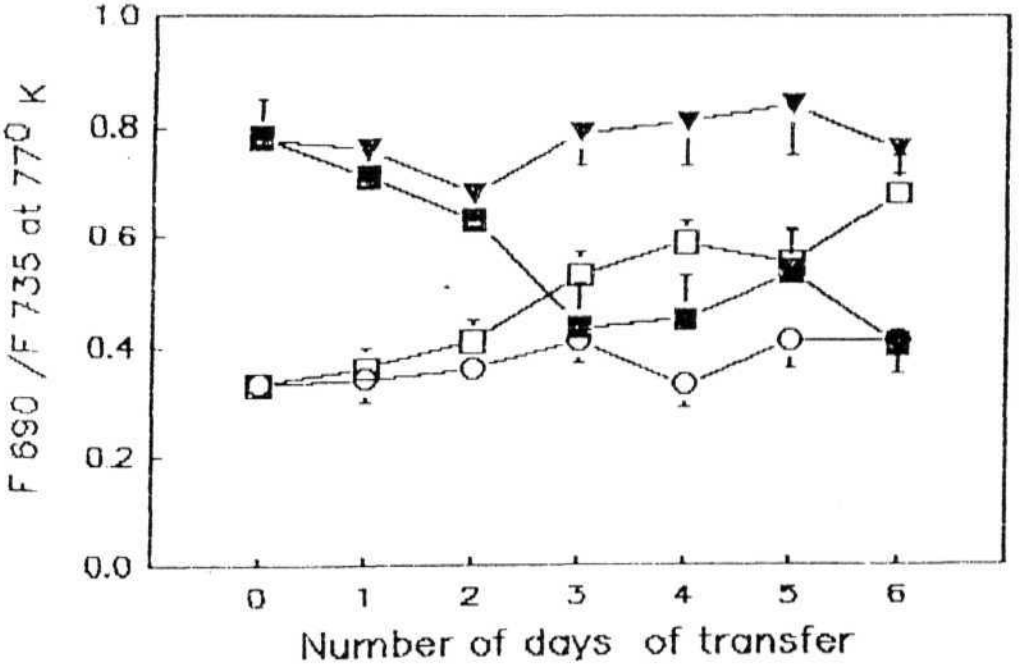

1.0
0.8
0.6
0.4
0.2
0.0
F 690 /F 735 at 77° K
0
1
2
3
4
5
6
Number of days of transfer

Figure 2.15a Effect of light intensity variations on F_{690}/F_{735} ratio at room temperature in mesophyll thylakoid membranes of *Amaranthus* .

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$

Figure 2.15b Effect of light intensity variations on F_{690}/F_{735}ratio at room temperature in mesophyll thylakoid membranes of *Amaranthus* .

0—0 H

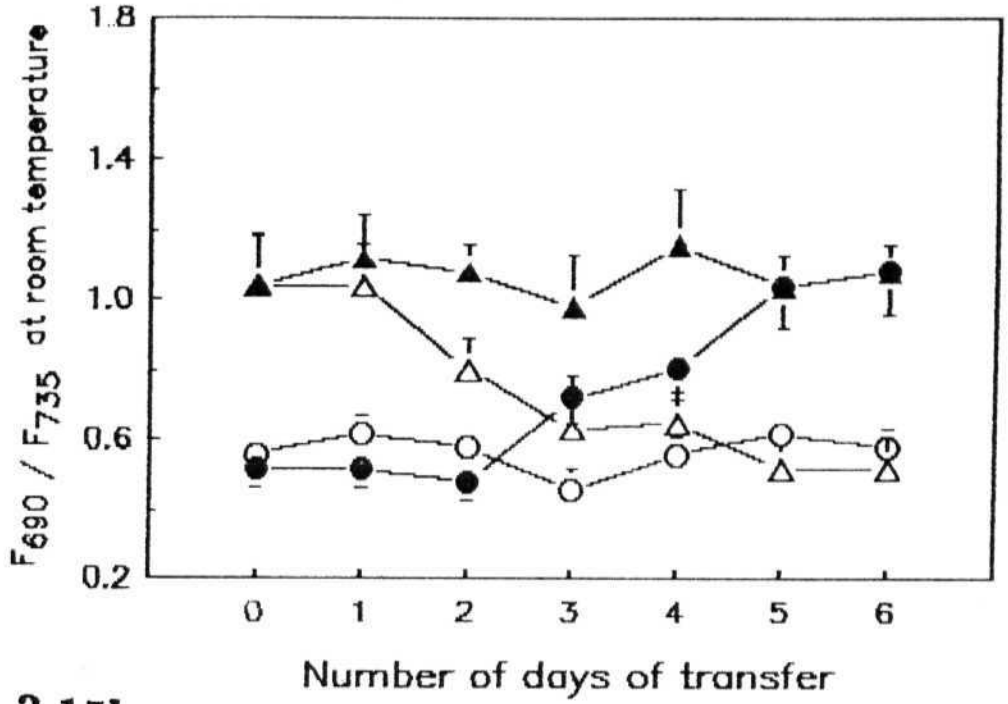
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three individual experiments.

2.15a


1.8
1.4
1.0
0.6
0.2
F_{690} / F_{735} at room temperature
0
1
2
3
4
5
6
Number of days of transfer

2.15b

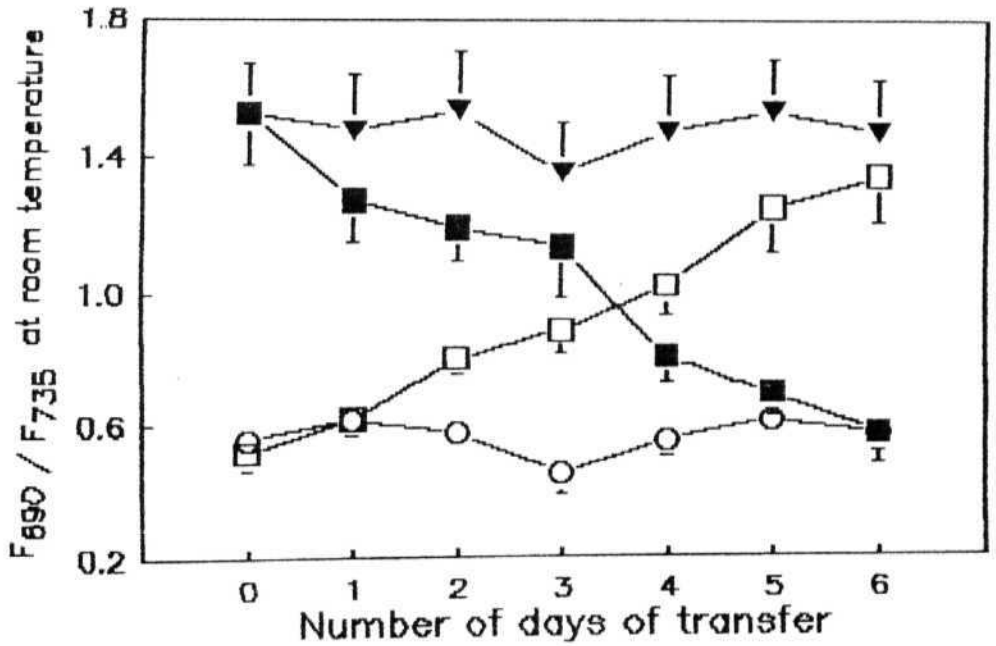

1.8
1.4
1.0
0.6
0.2
F_{690} / F_{735} at room temperature
0
1
2
3
4
5
6
Number of days of transfer

Figure 2.16a Effect of light intensity variations on F_{690}/F_{735} ratio at room temperature in bundle sheath thylakoid membranes of *Amaranthus*.

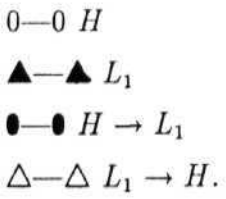
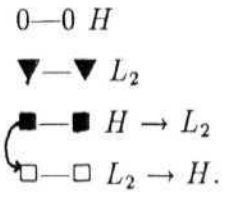
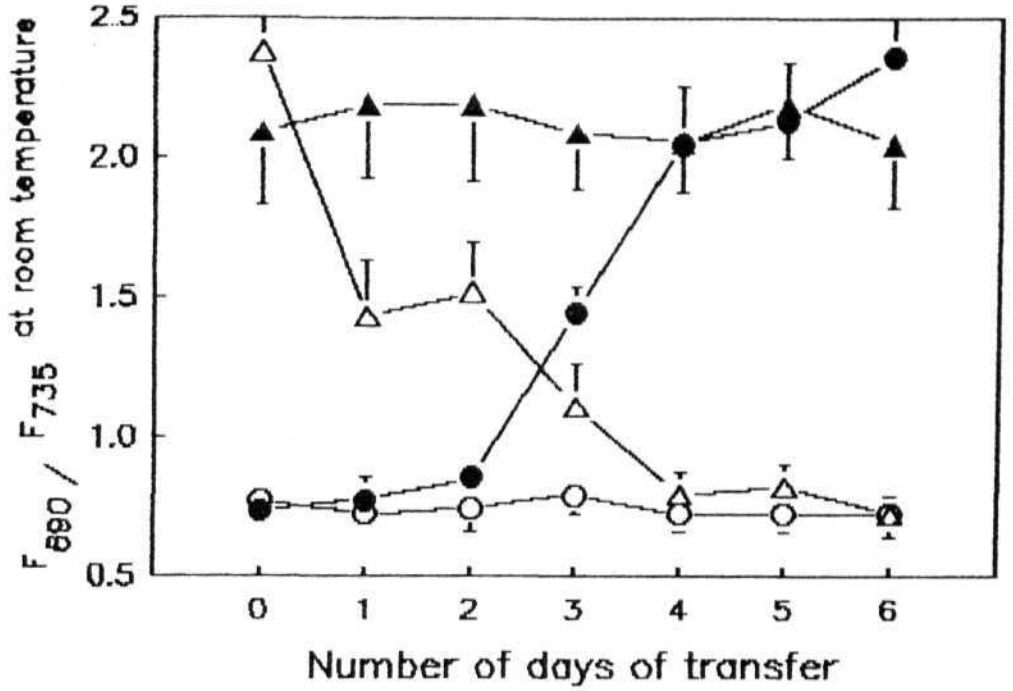


Figure 2.16b Effect of light intensity variations on F_{690}/F_{735} ratio at room temperature in bundle sheath thylakoid membranes of *Amaranthus.*

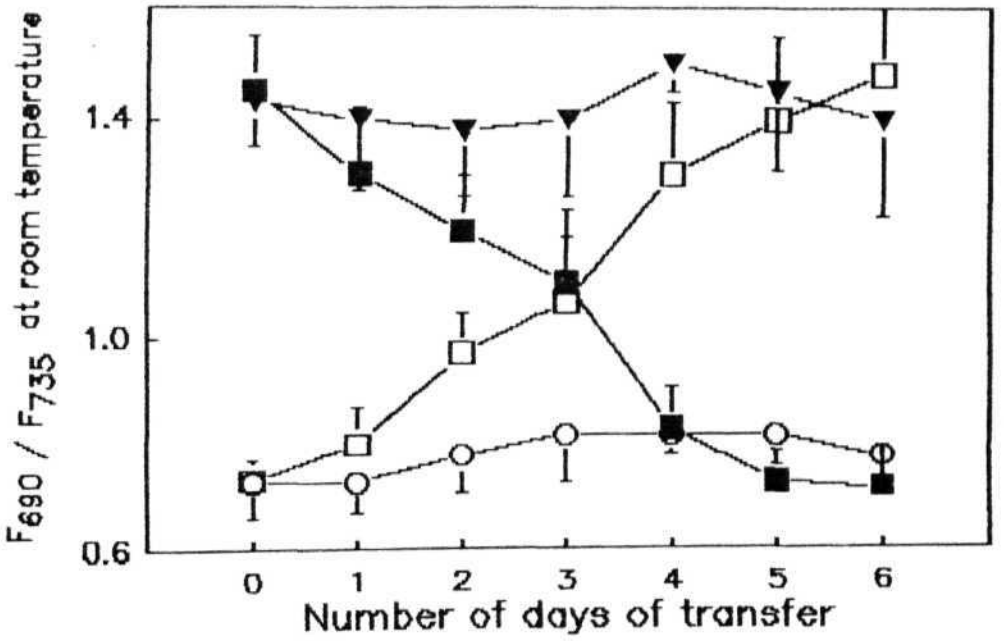


The results are average of three individual experiments.

2.16a


2.5
2.0
1.5
1.0
0.5
F690 / F735 at room temperature
0
1
2
3
4
5
6
Number of days of transfer

2.16b


1.4
1.0
0.6
F690 / F735 at room temperature
0
1
2
3
4
5
6
Number of days of transfer

plants showed 100% and 148% increase in F_{690}/F_{735} ratio at room temperature (Figures 2.15a, 2.15b, 2.16a and 2.16b). The alteration in the ratio in response to changes in irradiance was six days in both mesophyll and bundle sheath thylakoid membranes. The percentage change is higher in bundle sheath compared to that of mesophyll thylakoids when plants grown at one light were adjusted to different growth light regimes (Tables 2.1 and 2.2).

The F_{690}/F_{735} ratio at 77^{o}K increased by 113% and 165% in // $\rightarrow L_1$ and $H \rightarrow L_2$ plants of *Eleusine* whereas 52% and 67% reduction in the ratio was observed for $L\backslash \rightarrow H$ and $L_2 \rightarrow H$ *Eleusine* plants respectively (Figures 2.17a and 2.17b; Table 2.2). In $H \rightarrow L_1$ and // $\rightarrow L_2$ *Eleusine* plants F_{690}/F_{735} ratio at room temperature increased by 79% and 150% whereas the ratio decreased by 44% and 60% in $L_1 \rightarrow H$ and $L_2 \rightarrow H$ plants (Figures 2.18a and 2.18b). The changes in the ratios were observed in four and eight days, after an initial lag of twenty four hours. In *Gomphrena* $H \rightarrow L\backslash$ and $H \rightarrow L_2$ plants, F_{690}/F_{735} ratio increased by 70% and 121%, at 77^{o}K, whereas 77% and 123% increase was observed at room temperature respectively. However the ratio decreased in $L\backslash \rightarrow H$ and $L_2 \rightarrow H$ plants both at 77^{o}K (43%,56%) and at room temperature (41%,54%) (Figures 2.19a, 2.19b, 2.20a and 2.20b Tables 2.1 and 2.2). $H \rightarrow L_1$ and $L\backslash \rightarrow H$ plants took eight days to adjust the F_{690}/F_{735} ratio (both at 77^{o}K and room temperature) to altered environmental regimes whereas, $L_2 \rightarrow H$ and $H \rightarrow L_2$ plants exhibited variation in the ratio in ten days, with an initial lag of forty eight hours (Figures 2.19a, 2.19b, 2.20a and 2.20b Tables 2.1 and 2.2).

In $L_1 \rightarrow H$ and $L_2 \rightarrow H$ *Amaranthus* plants F_v/F_m increased by 125% and 200% at room temperature but only, 24% and 35% rise was observed at 77^{o}K respectively (Figures

Figure 2.17a Effect of light intensity variations on F_{690}/F_{735} **ratio at** 77^{o}K **in** *Eleusine coracana* thylakoid membranes.

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

Figure 2.17b Effect of light intensity variations on F_{690}/F_{735} ratio at 77^{o}K **in** *Eleusine coracana* thylakoid membranes.

0—0 H

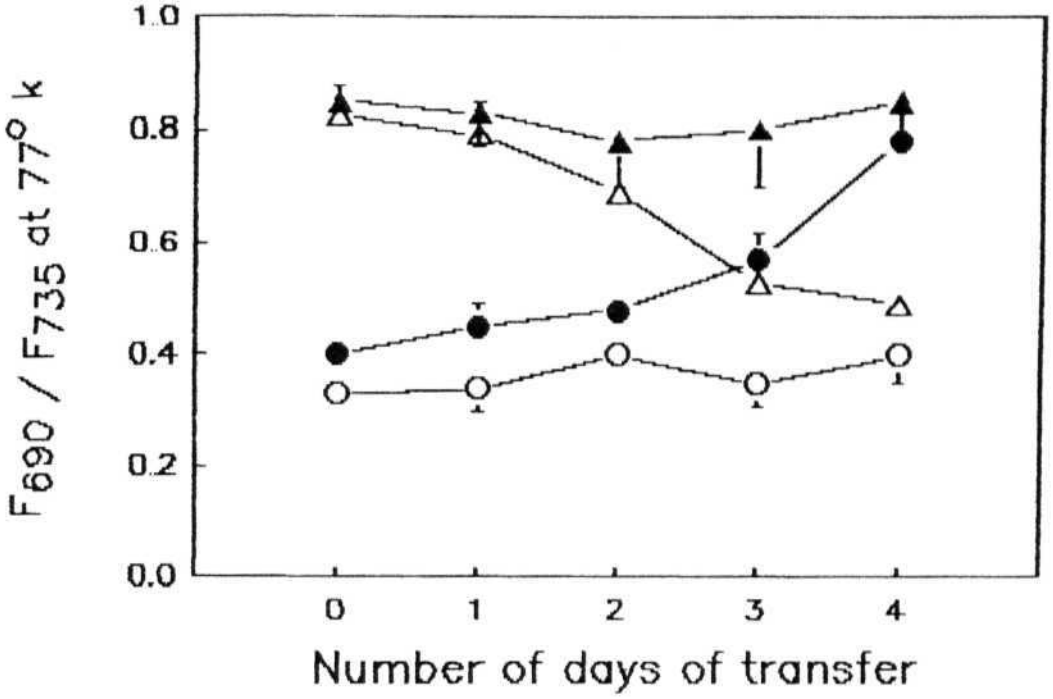
▼—▼ L_2

□—□ $H \rightarrow L_2$.

■—■ $L_2 \rightarrow H$

The results are average of three individual experiments.

2.17a


1.0
0.8
0.6
0.4
0.2
0.0
F_{690} / F_{735} at 77° k
0
1
2
3
4
Number of days of transfer

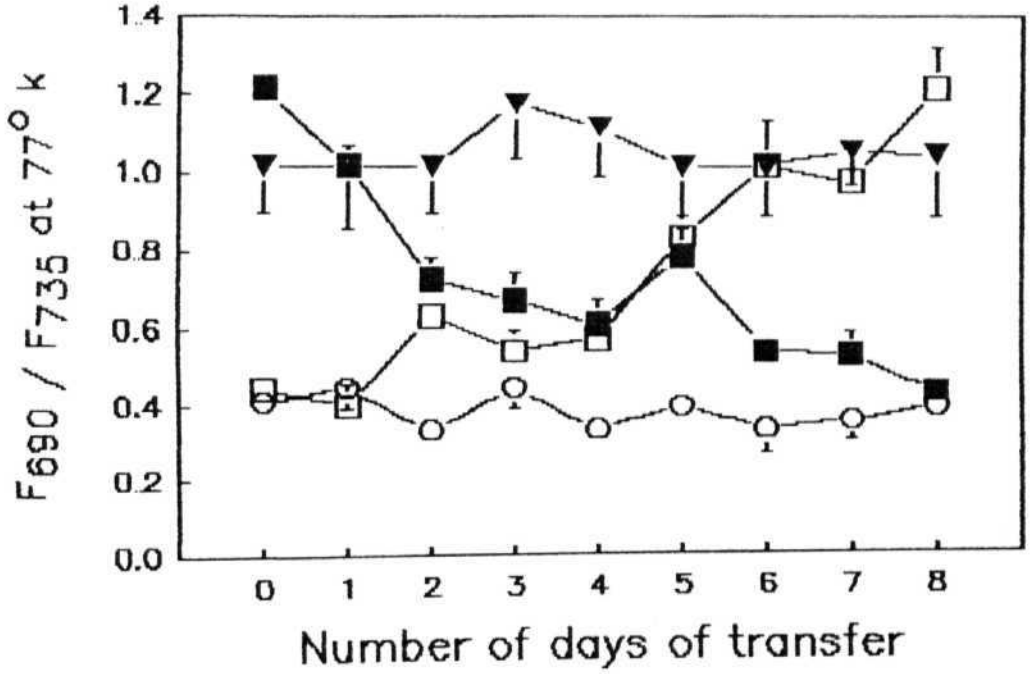

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
F_{690} / F_{735} at 77° k
0
1
2
3
4
5
6
7
8
Number of days of transfer

Figure 2.18a Effect of light intensity variations on F_{690}/F_{735} **ratio at** room **temperature** in *Eleusine coracana* thylakoid membranes.

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$

The results are average of three individual experiments.

Figure 2.18b Effect of light intensity variations on F_{690}/F_{735} ratio at room temperature in *Eleusine coracana* thylakoid membranes.

0—0 H

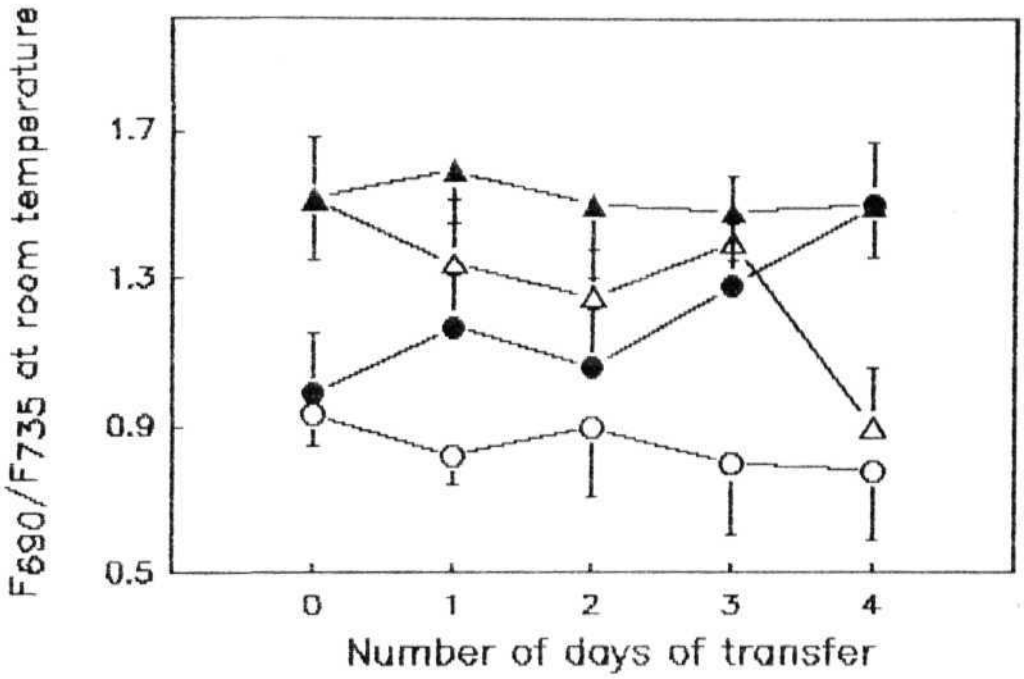
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

2.18a


F690/F735 at room temperature
1.7
1.3
0.9
0.5
0
1
2
3
4
Number of days of transfer

2.18b

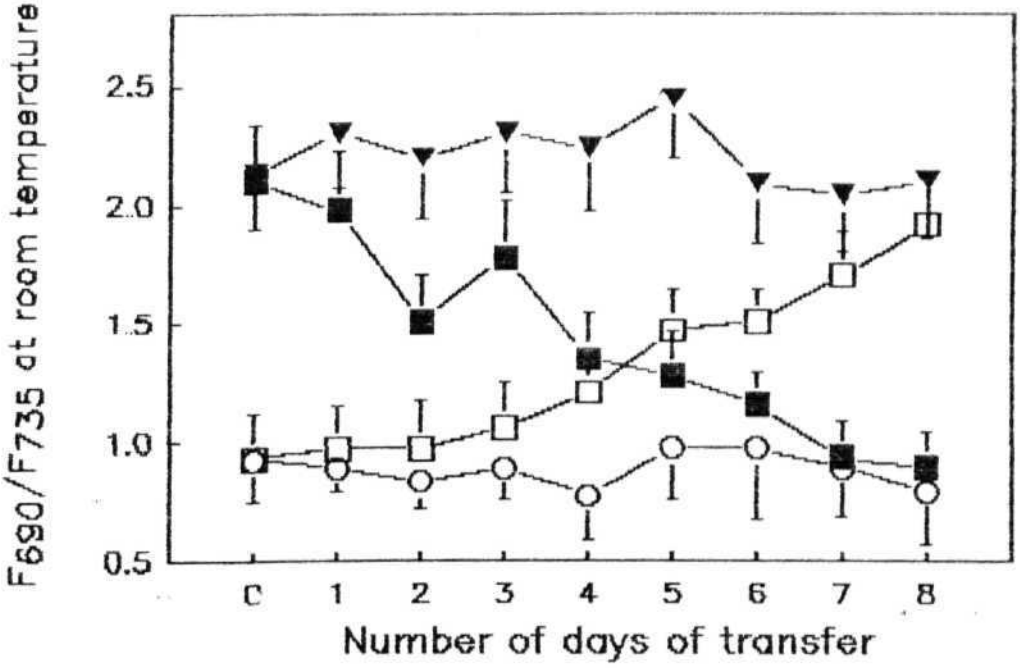

F690/F735 at room temperature
2.5
2.0
1.5
1.0
0.5
0
1
2
3
4
5
6
7
8
Number of days of transfer

Figure 2.19a Effect of light intensity variations on F_{690}/F_{735} ratio at 77°K **in** *Gomphrena globosa* thylakoid membranes.

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 2.19b Effect of light intensity variations on F_{690}/F_{735} ratio at 77°K in the *Gomphrena globosa* thylakoid membranes.

0—0 H

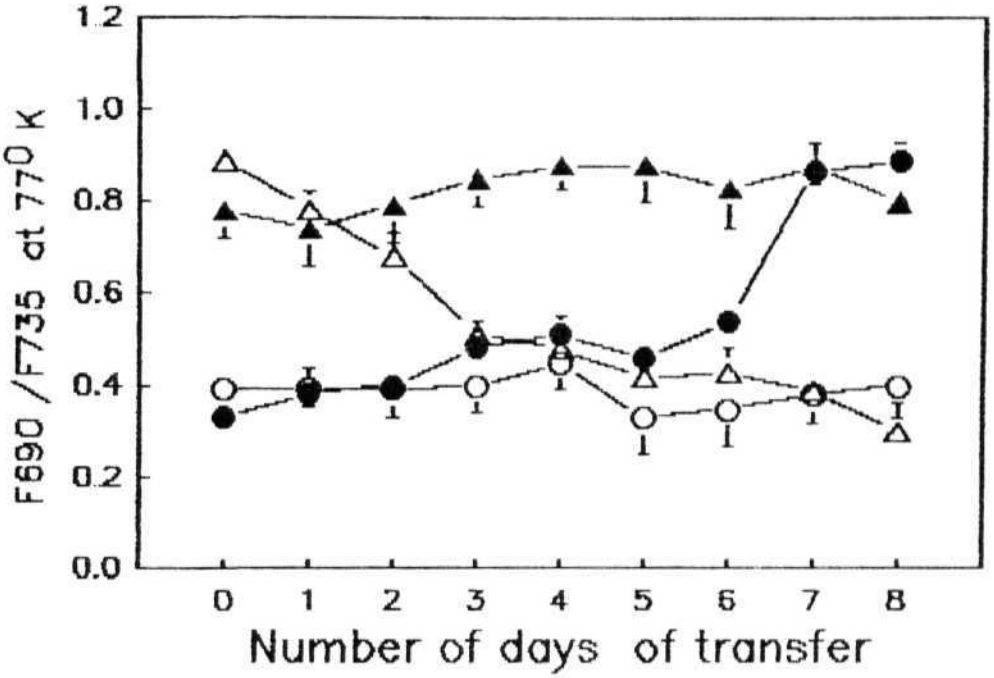
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

2.19a


F690 /F735 at 77^0 K
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8
Number of days of transfer

2.19b

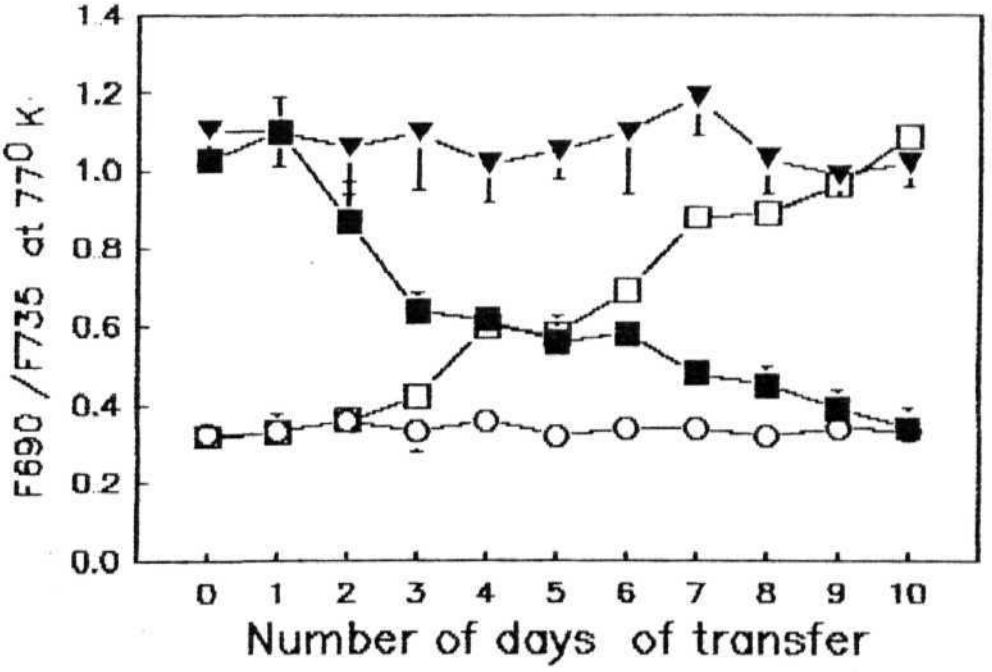

F690 /F735 at 77^0 K
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
Number of days of transfer

Figure 2.20a Effect of light intensity variations on F_{690}/F_{735} ratio at room temperature in *Gomphrena globosa.*

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 2.20b Effect of light intensity variations on F_{690}/F_{735} ratio at room temperature in *Gomphrena globosa.*

0—0 H

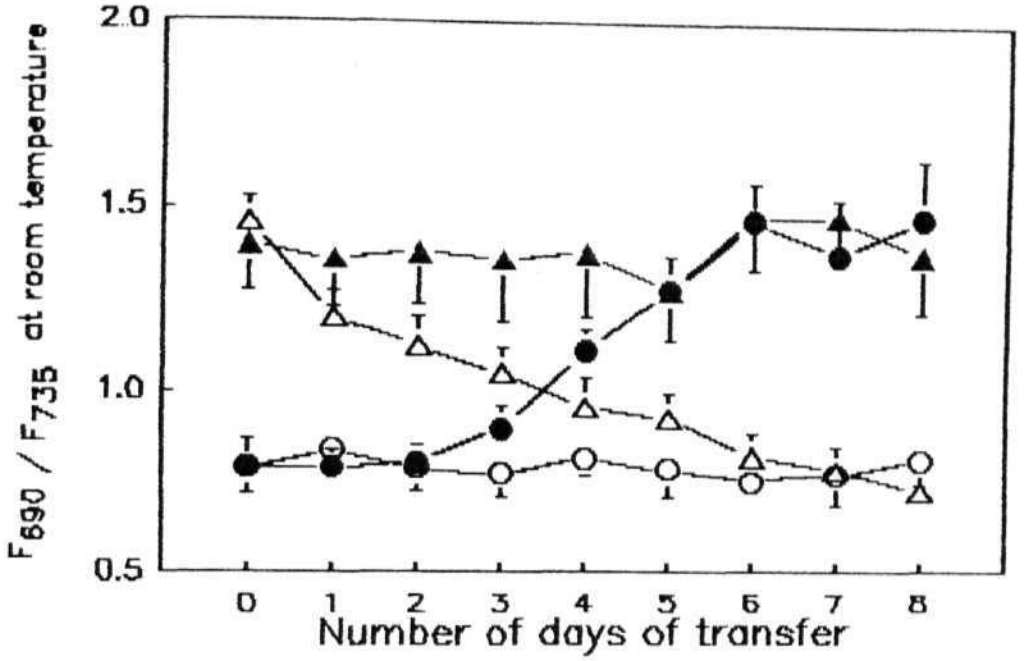
▼—▼ L_2

□—□ $H \rightarrow L_2$

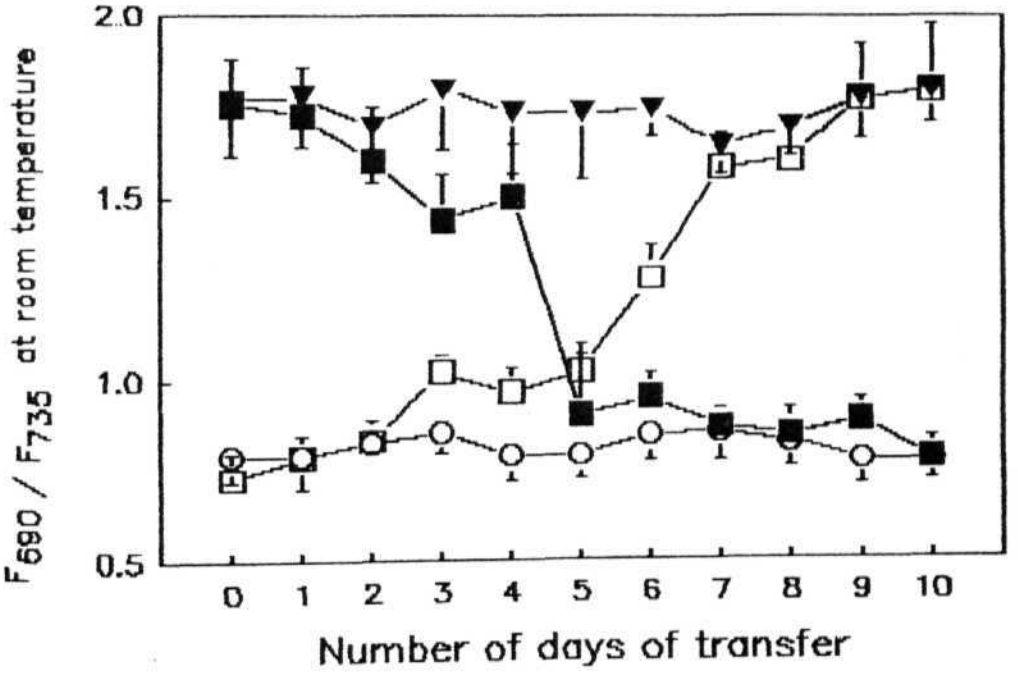
■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

2.20a


2.0
1.5
1.0
0.5
F_{690} / F_{735} at room temperature
0 1 2 3 4 5 6 7 8
Number of days of transfer

2.20b


2.0
1.5
1.0
0.5
F_{690} / F_{735} at room temperature
0 1 2 3 4 5 6 7 8 9 10
Number of days of transfer

2.21a, 2.21b, 2.22a and 2.22b Tables 2.3 and 2.4). $H \rightarrow L_1$ and $H \rightarrow L_2$ plants showed 62% and 66% decrease in the ratio at room temperature while 19% and 24% decrease was observed at 77^{o}K respectively in a time span of six days (Figures 2.21a, 2.21b, 2.22a and 2.22b Tables 2.3 and 2.4). $L_1 \rightarrow H$ and $L_2 \rightarrow H$ plants of *Eleusine* exhibited 121% and 183% increase at room temperature but only 13% and 33% increase was observed at 77^{o}K respectively (Figures 2.23a, 2.23b 2.24a and 2.24b Tables 2.3 and 2.4). $H \rightarrow L_1$ and $H \rightarrow L_2$ *Eleusine* plants showed 54% and 65% reduction in F_v/F_m at room temperature, but only 11% and 25% reduction was observed in the ratio at 77^{o}K respectively (Figures 2.23a, 2.23b, 2.24a and 2.24b Tables 2.3 and 2.4). The $L_1 \rightarrow H$ and $L_2 \rightarrow H$ *Gomphrena* plants showed 115% and 164% increase in F_v/F_m at room temperature, while 12% and 24% increase in the ratio was observed at 77^{o}K (Figures 2.25a, 2.25b, 2.26a and 2.26b Tables 2.3 and 2.4). F_v/F_m ratio decreased in $H \rightarrow L_1$ and $H \rightarrow L_2$ plants (53% and 62% at room temperature and 10% and 20% reduction at 77^{o}K). The alteration in the ratio in response to changes in irradiance was manifested after an initial lag of forty eight hours in both *Eleusine* and *Gomphrena.* The modulation in F_v/F_m ratio in response to reduced growth light, (at both room temperature and at 77^{o}K) was observed in four and eight days respectively, in *Eleusine*, whereas in *Gomphrena* it was much longer, eight and ten days respectively (Figures 2.23a, 2.23b, 2.24a, 2.24b, 2.25a, 2.25b, 2.26a and 2.26b). *Gomphrena* showed least reduction in F_v/F_m under suboptimal light (at both room temperature and at 77^{o}K), whereas *Amaranthus* showed highest reduction in the ratio (Tables 2.3 and 2.4).

Electron transport rates:

Whole chain electron transport rates decreased by 64% of the control (H) in the mesophyll thylakoid membranes of L_I *Amaranthus* plants (Table 3.1). While the same

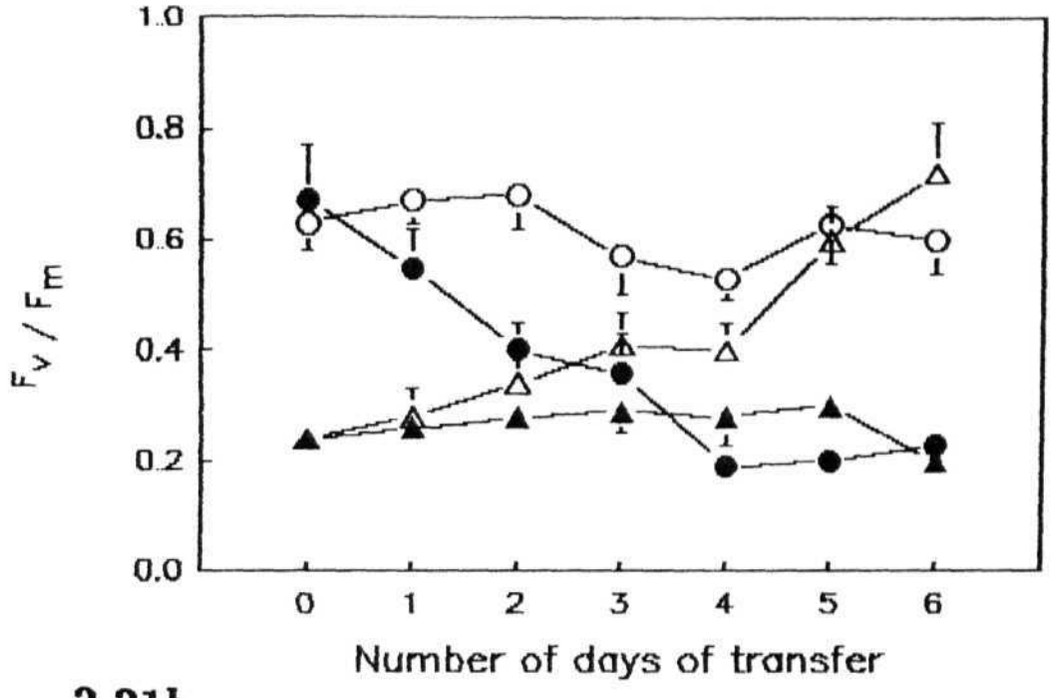
Figure 2.21a Fluorescence transients at room temperature in leaves of *Amaranthus hypochondriacus* L. under different light intensities.

The results are average of ten independent values.

Figure 2.21b Fluorescence transients at room temperature in leaves of *Amaranthus hypochondriacus* L. under different light intensities.

The results are average of ten independent values.

2.21a


1.0
0.8
0.6
0.4
0.2
0.0
F_v / F_m
0
1
2
3
4
5
6
Number of days of transfer

2.21b

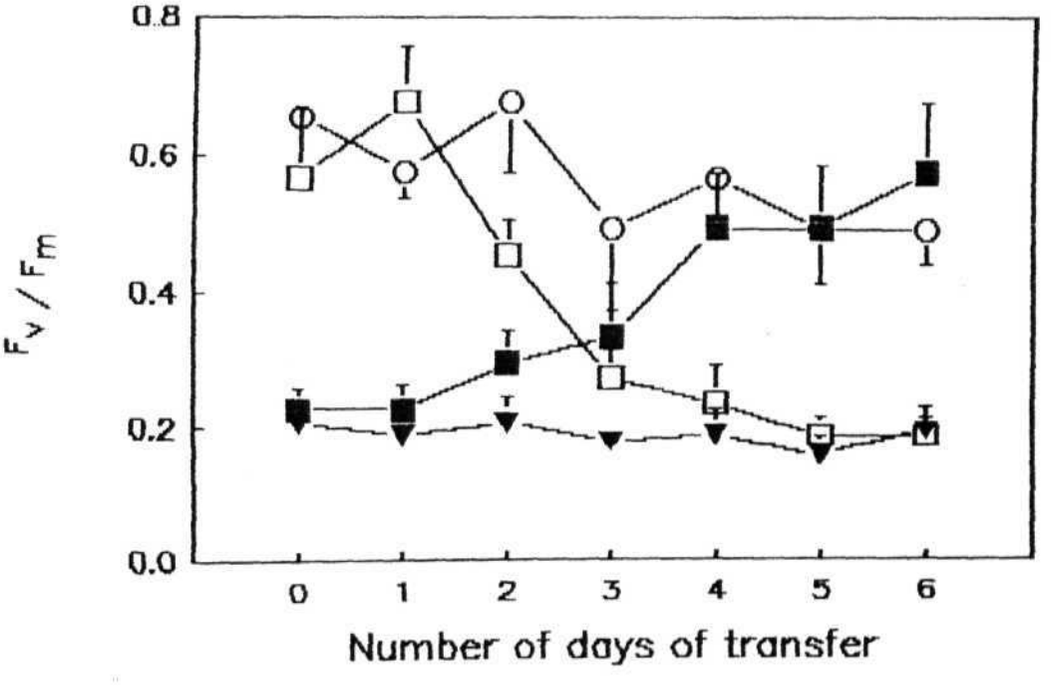

0.8
0.6
0.4
0.2
0.0
F_v / F_m
0
1
2
3
4
5
6
Number of days of transfer

Figure 2.22a Fluorescence transients at 77^{o}K in the leaves of *Amaranthus hypochondriacus* L. under different light regimes.

0—0 H

▲—▲ L_1

⬮—⬮ $H \rightarrow L_1$.

△—△ $L_1 \rightarrow H$

The results are average of ten independent values.

Figure 2.22b Fluorescence transients at 77^{o}K in the leaves of *Amaranthus hypochondriacus* L. under different light regimes.

0—0 H

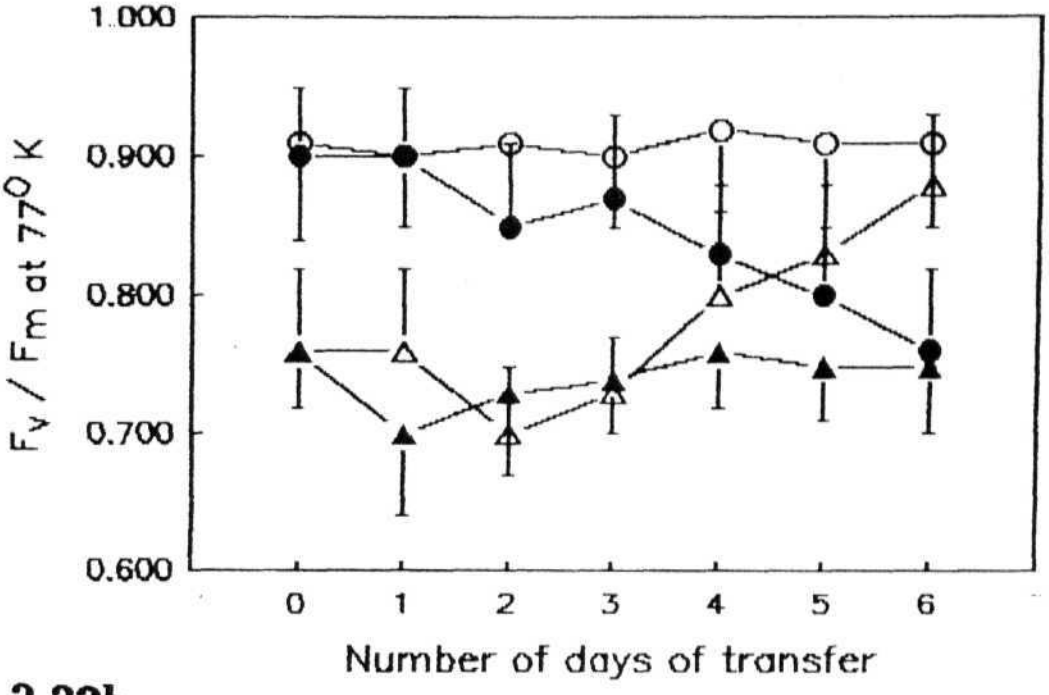
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of ten independent values.

2.22a


1.000
0.900
0.800
0.700
0.600
F_v / Fm at 77^0 K
0
1
2
3
4
5
6
Number of days of transfer

2.22b

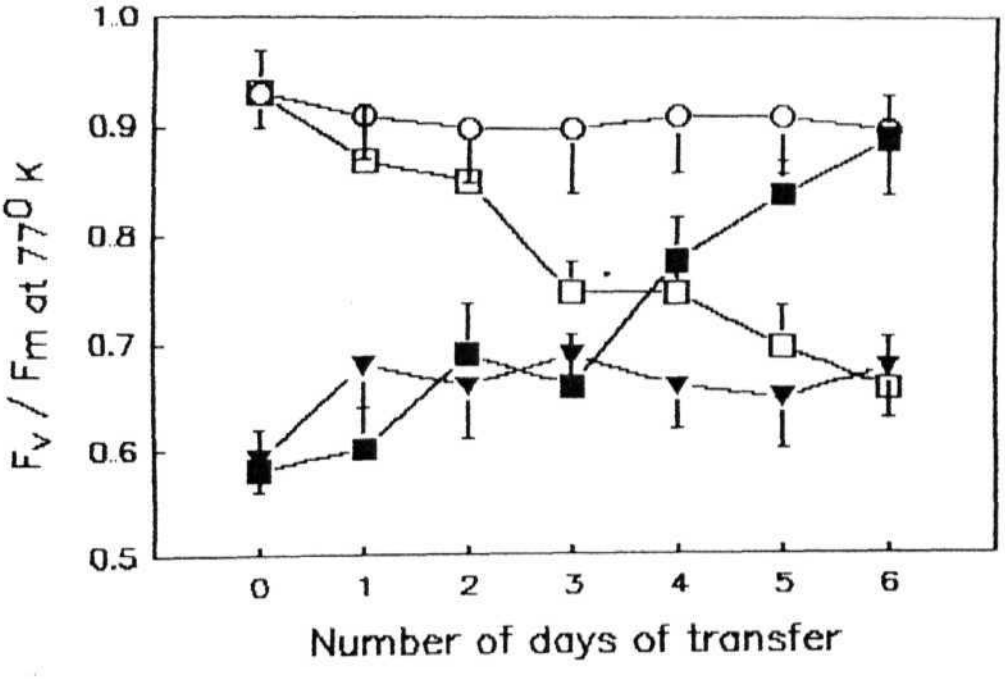

1.0
0.9
0.8
0.7
0.6
0.5
F_v / Fm at 77^0 K
0
1
2
3
4
5
6
Number of days of transfer

Figure 2.23a Fluorescence transients at room temperature in the leaves of *Eleusine coracana* under different light regimes.

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of ten independent values.

Figure 2.23b Fluorescence transients at room temperature in the leaves of *Eleusine coracana* under different light regimes.

0—0 H

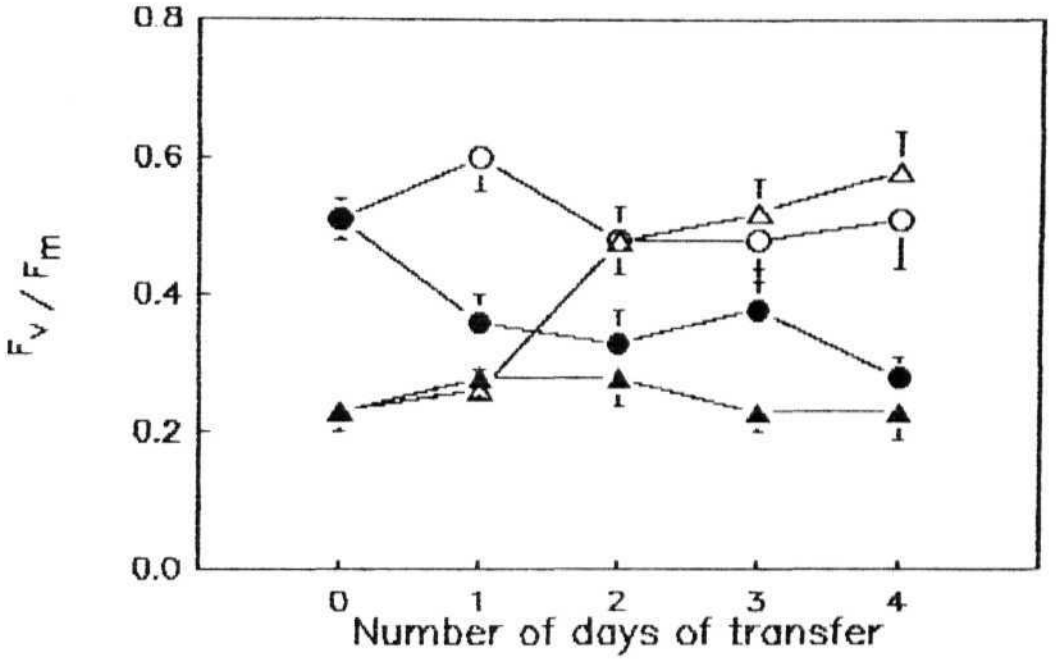
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of ten independent values.

2.23a


0.8
0.6
0.4
0.2
0.0
F_v / F_m
0
1
2
3
4
Number of days of transfer

2.23b

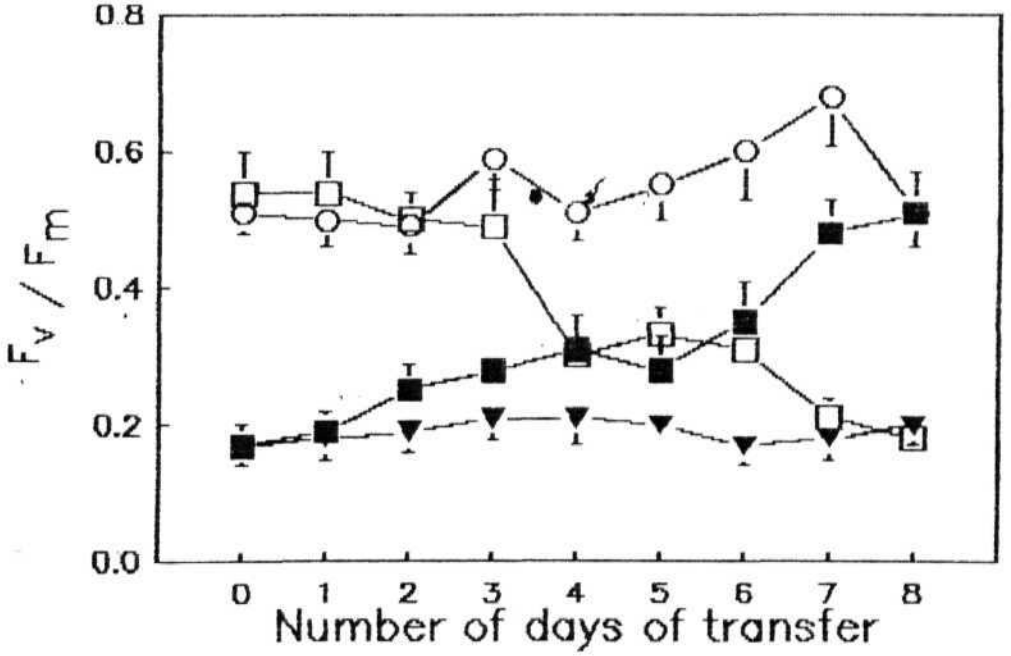

0.8
0.6
0.4
0.2
0.0
F_v / F_m
0
1
2
3
4
5
6
7
8
Number of days of transfer

Figure 2.24a. Effect of light intensity on fluorescence transients at 77°K in the leaves of *Eleusine coracana.*

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of ten independent values.

Figure 2.24b Effect of light, intensity on fluorescence transients at 77°K in the leaves of *Eleusine coracana.*

0—0 H

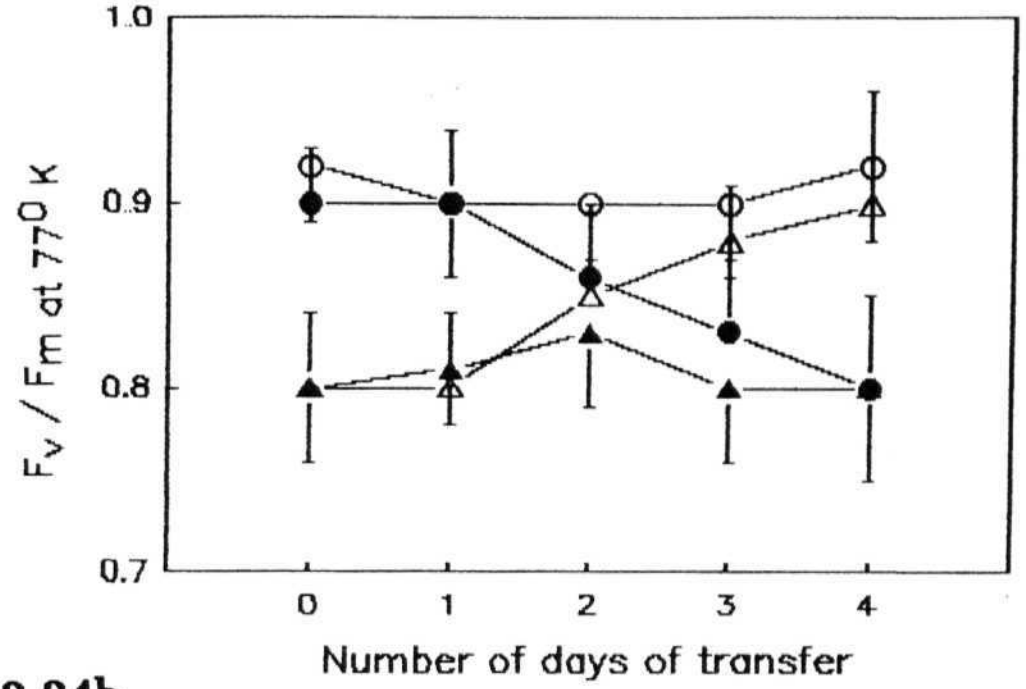
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$

The results are average of ten independent values.

2.24a


1.0
0.9
0.8
0.7
F_v / Fm at 77^0 K
0
1
2
3
4
Number of days of transfer

2.24b

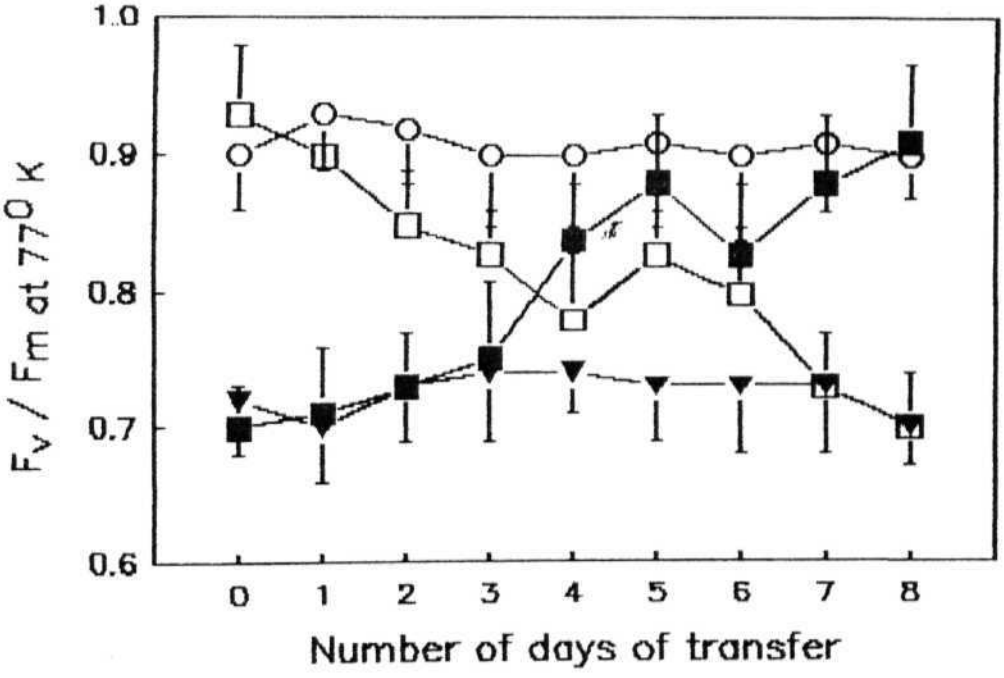

1.0
0.9
0.8
0.7
0.6
F_v / Fm at 77^0 K
0
1
2
3
4
5
6
7
8
Number of days of transfer

Figure 2.25a Fluorescence transients at room temperature **in the leaves of** *Gomphrena globosa* under different light regimes.

0—0 H

▲—▲ L_1

⬮—⬮ $H \rightarrow L_1$.

△—△ $L_1 \rightarrow H$

The results are average of ten independent values.

Figure 2.25b Fluorescence transients at room temperature in the leaves of *Gomphrena globosa* under different light regimes.

0—0 H

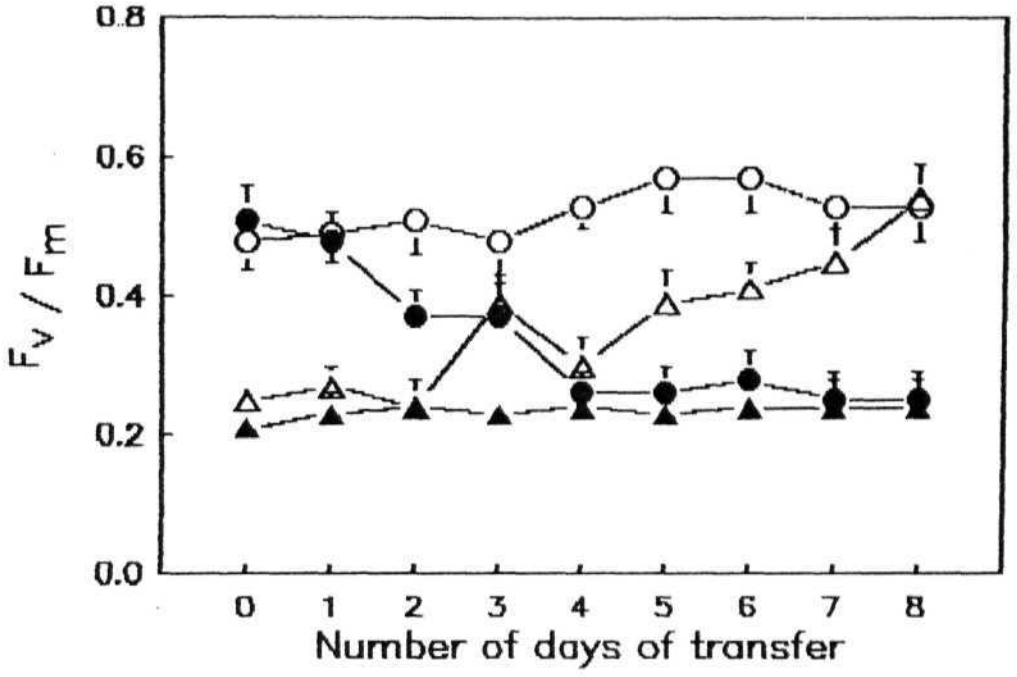
▼—▼ L_2

□—□ $H \rightarrow L_2$.

■—■ $L_2 \rightarrow H$

The results are average of ten independent values.

2.25a


0.8
0.6
0.4
0.2
0.0
F_v / F_m
0 1 2 3 4 5 6 7 8
Number of days of transfer

2.25b

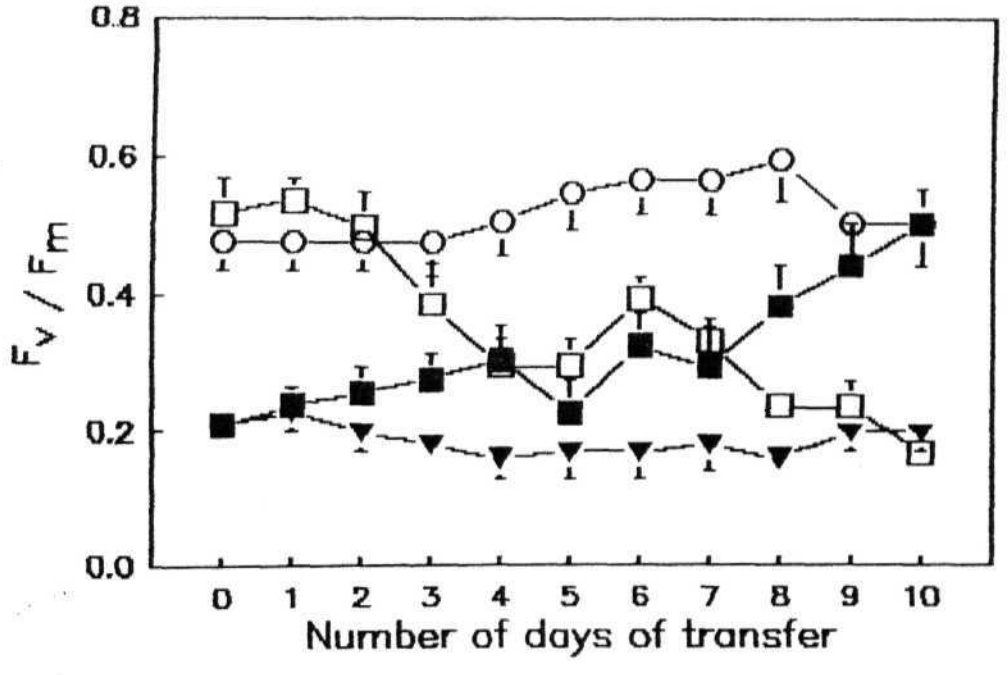

0.8
0.6
0.4
0.2
0.0
F_v / F_m
0 1 2 3 4 5 6 7 8 9 10
Number of days of transfer

Figure 2.26a Fluorescence transients at 77°K in *Gomphrena globosa* under various light regimes.

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$.

△—△ $L_1 \rightarrow H$.

The results are average of ten independent values.

Figure 2.26b Fluorescence transients at 77°K in the leaves of *Gomphrena globosa* under various light regimes.

0—0 H

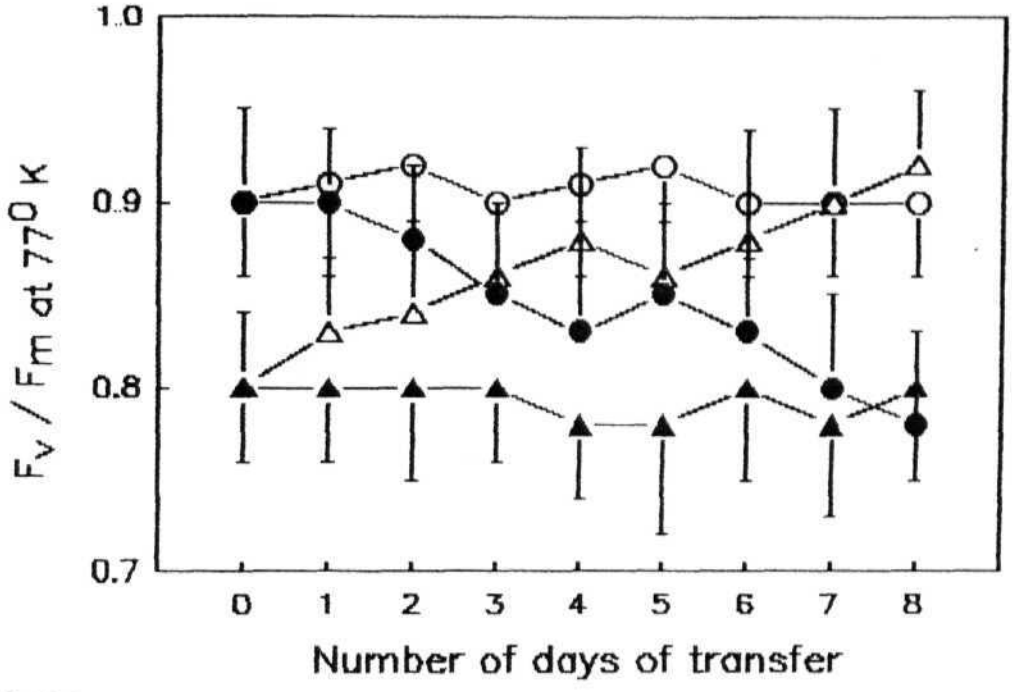
▼—▼ L_2

□—□ $H \rightarrow L_2$

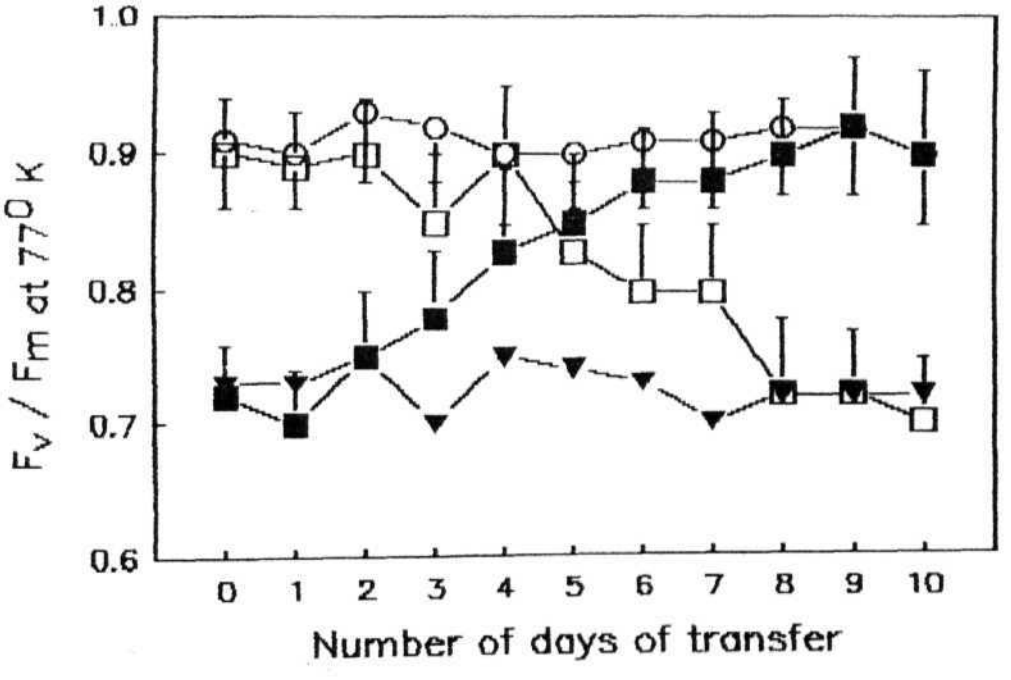
■—■ $L_2 \rightarrow H$.

The results are average of ten independent values.

2.26a


1.0
0.9
0.8
0.7
F_v / Fm at 77^0 K
0 1 2 3 4 5 6 7 8
Number of days of transfer

2.26b


1.0
0.9
0.8
0.7
0.6
F_v / Fm at 77^0 K
0 1 2 3 4 5 6 7 8 9 10
Number of days of transfer

decreased by 58% of the control values in the bundle sheath thylakoid membranes of L_I plants. The whole chain electron transport rates decreased by 73% and 67% of the control values in the mesophyll and bundle sheath thylakoid membranes of L_2 plants respectively. The reduction in the whole chain electron transport rates in general was lesser in bundle sheath compared to mesophyll at fixed reduction in growth light intensity.

The whole chain electron transport rates decreased by 57% and 54% of the high light control values in mixed thylakoid membranes of $L\backslash$ plants of *Eleusine* and *Gomphrena* respectively (Table 3.1). The whole chain electron transport rates of L_2 plants of *Eleusine* and *Gomphrena* decreased by 72% and 65% of the (H) rates respectively (Table 3.1). *Gomphrena* plants grown at reduced intensities showed maximum decrease in the whole chain electron transport. The electron transport decreased significantly under lowered light in each of the plants studied.

PSI electron transport rates were reduced by 47% and 44% in mesophyll and bundle sheath thylakoids of L_1 plants and 75% and 63% in L_2 plants of *Amaranthus* respectively (Tables 3.2). The impairment in PSI electron transport in response to reduced growth irradiance was also observed in *Eleusine* and *Gomphrena* plants. In the L_1 plants of *Eleusine* and *Gomphrena* PSI electron transport decreased by 63% and 52% of H plants respectively. The L_2 plants showed 77% and 73% of decreased rates respectively in *Eleusine* and *Gomphrena* (Table 3.2)>

PSII electron transport was also sensitive to reduced growth irradiances. The PSI1 electron transport decreased by 67% and 62% in mesophyll and bundle sheath thylakoids of L_1 plants in *Amaranthus* (Table 3.3)、 The decrease in PSII electron transport was 86% and 81% of the highlight control in mesophyll and bundle sheath thylakoids of L_2

Table 3.1. Effect of reduced irradiance on whole clain electron transport.

Plant Species	Whole claim electron transport (rates μmoles mgchl^{-1}h^{-1})			% decrease of control on acclimation		% increase of control on acclimation	
	H	L_1	L_2	H→L_1	H→L_2	L_1→H	L_2-H
A. hypochondriacus							
mesophyll	1247±202	448±56[a]	334±44[a]	64.0	73.2	178	273
bundle sheath	1086±186	447±64[a]	364±58[a]	58.0	66.6	142	198
E. coracana	960±136	417±71[b]	250±28[b]	56.5	72.0	130	256
G. globosa	942±148	432±48[b]	331±48[b]	54.0	64.8	118	184

The results are average of three individual experiments.

H : Thylakoid membranes from high light (2000 μE m^{-2}s^{-1}) grown plants.

L_1: Thylakoid membranes from low light (650 μE m^{-2}s^{-1}) grown plants.

L_2: Thylakoid membranes from low light (200 μE m^{-2}s^{-1}) grown plants.

L_1→H: Thylakoid membranes from low light (650 μE m^{-2}s^{-1}) grown plants after acclimation to high irradiance (2000 μE m^{-2}s^{-1}).

L_2→H: Thylakoid membranes from low light (200 μE m^{-2}s^{-1}) grown plants acclimated to high irradiance (2000 μE m^{-2}s^{-1}).

H→L_1: Thylakoid membranes from high irradiance (2000 μE m^{-2}s^{-1}) grown plants acclimated to reduced light (650 μE m^{-2}s^{-1}).

H→L_2: Thylakoid membranes from high irradiance (2000 μE m^{-2}s^{-1}) grown plants acclimated to reduced light (200 μE m^{-2}s^{-1}).

a = $p < 0.001$ b = $p < 0.005$

Table 3.2. Effect of reduced irradiance on PSI electron transport.

Plant Species	PSI electron transport rates (μmoles mgchl^{-1}h^{-1})			% decrease of control on acclimation		% increase of control on acclimation	
	H	L_1	L_2	H→L_1	H→L_2	L_1→H	L_2-H
A. hypochondriacus							
mesophyll	2166±165	1146±171[a]	533±38[a]	47.0	75	273	306
bundle sheath	2235±285	1242±187[a]	843±98[a]	44.0	63	165	198
E. coracana	2827±434	1038±148[b]	637±120[a]	63.2	77	256	343
G. globosa	2142±178	1018±138[a]	556±105[a]	52.4	73	184	285.

The results are average of three individual experiments.

H : Thylakoid membranes from high light (2000 μE m^{-2}s^{-1}) grown plants.

L_1: Thylakoid membranes from low light (650 μE m^{-2}s^{-1}) grown plants.

L_2: Thylakoid membranes from low light (200 μE m^{-2}s^{-1}) grown plants.

L_1→H: Thylakoid membranes from low light (650 μE m^{-2}s^{-1}) grown plants after acclimation to high irradiance (2000 μE m^{-2}s^{-1}).

L_2→H: Thylakoid membranes from low light (200 μE m^{-2}s^{-1}) grown plants acclimated to high irradiance (2000 μE m^{-2}s^{-1}).

H→L_1: Thylakoid membranes from high irradiance (2000 μE m^{-2}s^{-1}) grown plants acclimated to reduced light (650 μE m^{-2}s^{-1}).

H→L_2: Thylakoid membranes from high irradiance (2000 μE m^{-2}s^{-1}) grown plants acclimated to reduced light (200 μE m^{-2}s^{-1}).

a = $p < 0.001$ b = $p < 0.005$.

Table 3.3. Effect of reduced irradiance on PSII electron transport.

Plant Species	PSII electron transport rates (μmoles mgchl^{-1}h^{-1})			% decrease of control on acclimation		% increase of control on acclimation	
	H	L_1	L_2	H→L_1	H→L_2	L_1→H	L_2-H
A. hypochondriacus							
mesophyll	574±54	188±16[a]	82±16[a]	67	86	205	600
bundle sheath	534±66	202±18[a]	96±18[a]	62	81	164	456
E. coracana	184±19	53±12[a]	24±16[a]	71	86	247	660
G. globosa	142±20	28±16[a]	28±6[a]	61	81	160	439

The results are average of three individual experiments.

H : Thylakoid membranes from high light (2000 μE $m^{-2}s^{-1}$) grown plants.

L_1: Thylakoid membranes from low light (650 μE $m^{-2}s^{-1}$) grown plants.

L_2: Thylakoid membranes from low light (200 μE $m^{-2}s^{-1}$) grown plants.

L_1→H: Thylakoid membranes from low light (650 μE $m^{-2}s^{-1}$) grown plants after acclimaiion to high irradiance (2000 μE $m^{-2}s^{-1}$).

L_2→H: Thylakoid membranes from low light (200μE $m^{-2}s^{-1}$)grownplantsacclimated to high irradiance (2000 μE $m^{-2}s^{-1}$).

H→L_1: Thylakoid membranes from high irradiance (2000μE $m^{-2}s^{-1}$) grown plants acclimated to reduced light (650 μE $m^{-2}s^{-1}$).

H→L_2: Thylakoid membranes from high irradiance (2000 μE $m^{-2}s^{-1}$) grown plants acclimated to reduced light (200 μE $m^{-2}s^{-1}$).

a = $p < 0.001$

plants in *Amaranthus* (Table 3.3). PS1I electron transport rates decreased to a greater extent compared to PSI electron transport rates under fixed reduced irradiances of one third and one tenth growth light regimes.

Eleusine and *Gomphrena* plants grown under suboptimal light (L_1 and L_2) also showed greater reduction in PSII electron transport compared to that of PSI. (Tables 3.2 and 3.3). The L_1 and L_2 plants of *Eleusine* showed 71% and 86% reduction respectively. In *Gomphrena* L_1 and L_2 plants, 61% and 81% decrease in PSII electron transport was observed compared to highlight grown plants. Out of the three plants studied *Gomphrena* showed least reduction in the electron transport rates in response to reduced irradiances (Table 3.3).

Amaranthus plants grown under high light on transfer to reduced irradiance showed decreased whole chain electron transport rates without initial lag. The electron transport rates decreased in mesophyll thylakoids of $H \rightarrow L$\ plants by 64% of the H plants and reached the rates of L_1 plants in a time period of six days (Table 3.1). The $L_1 \rightarrow H$ plants on transfer to high irradiance showed 178% increased whole chain electron transport in a time span of six days (Figure 3.1a and Table 3.1). However, only 58% decrease was observed in $H \rightarrow L_1$ bundle sheath thylakoids. The transport increased by 142% compared to that of L_1 plants when $L_1 \rightarrow H$ plants were transferred. (Table 3.1). The whole chain electron transport of $L_2 \rightarrow H$ plants of *A maranthus* decreased by 72% in the mesophyll thylakoids. The transport increased by 273% of the L_2 when $L_2 \rightarrow H$ plants were transferred to high light intensity (Figure 3.1b). The $H \rightarrow L_2$ *Amaranthus* plants on transfer to lowered irradiance showed 66% decreased whole chain electron transport in the bundle sheath thylakoids while $L_2 \rightarrow H$ plants showed 198% increase in the transport

Figure 3.1a. Effect of light intensity on whole chain electron transport rates in the mesophyll thylakoid membranes of *Amaranthus hypochondriacus* L.

0—0 H

▲—▲ L_1

•—• $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 3.1b Effect of light intensity on whole chain electron transport rates in the mesophyll thylakoid membranes of *Amaranthus hypochondriacus* L.

0—0 H

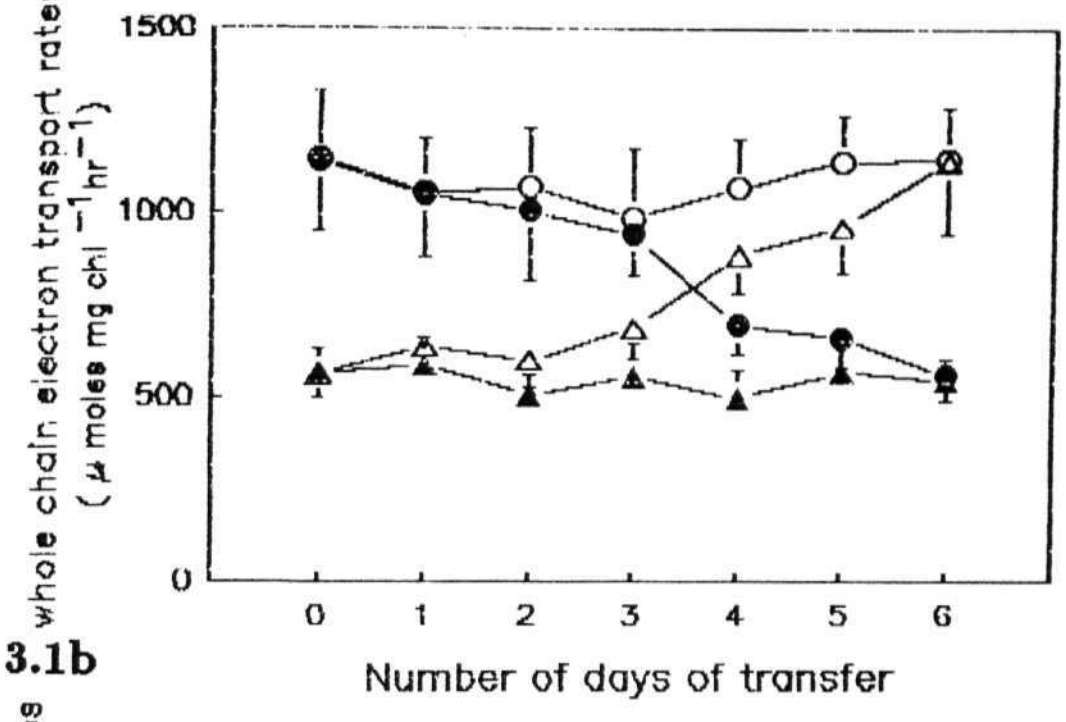
▼—▼ L_2

□—□ $H \rightarrow L_2$

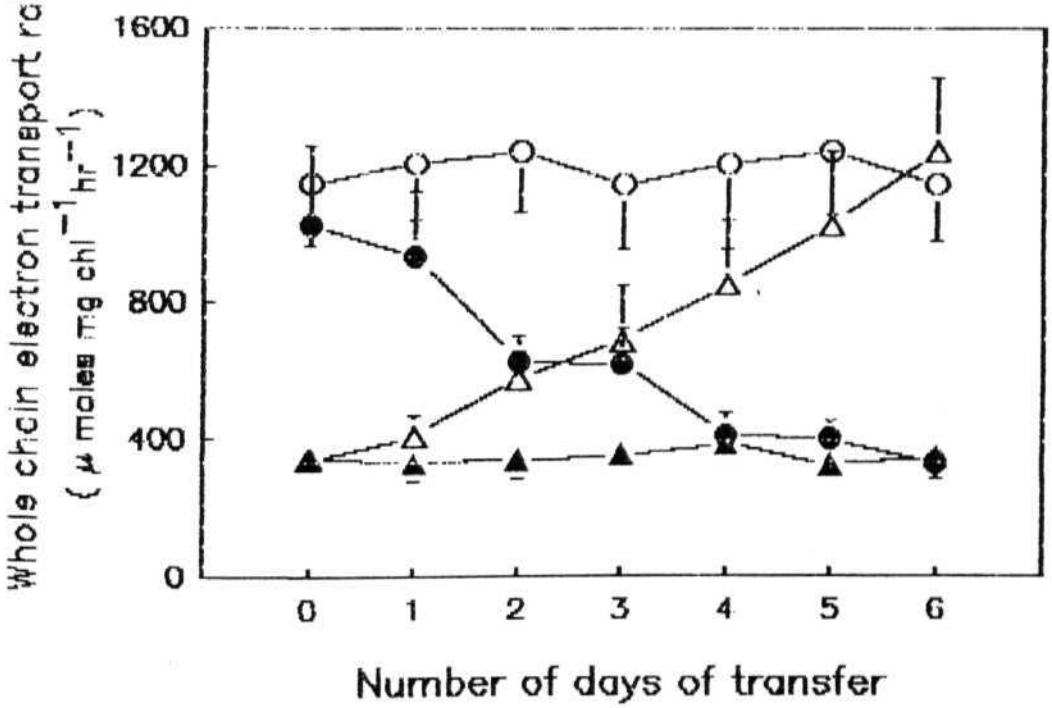
■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

3.1a


Whole chain electron transport rates
(μ moles mg chl $^{-1}$hr^{-1})
1500
1000
500
0
0
1
2
3
4
5
6
Number of days of transfer

3.1b


Whole chain electron transport rates
(μ moles mg chl $^{-1}$hr^{-1})
1600
1200
800
400
0
0
1
2
3
4
5
6
Number of days of transfer

when these plants were transferred to high light intensity (Figure 3.2b). The increase or decrease in the electron transport of mesophyll and bundle sheath thylakoids after the transfer to different light regimes was not linear during the time course of acclimation (Figures 3.1a, 3.1b, 3.2a and 3.2b). The time taken for the modulation of whole chain electron transport in response to changes in light intensities was six days in both mesophyll and bundle sheath thylakoid membranes. The mesophyll thylakoid membranes showed greater reduction in the whole chain electron transport in *Amaranthus*, compared to that of bundle sheath at suboptimal irradiances (Table 3.1).

PSI and PSII electron transports were found to be sensitive to transfer of plants from one light regime to another in *Amaranthus*. When $H \rightarrow L_1$ plants were transferred to reduced irradiance the PSI electron transport in mesophyll decreased by 47% of *H* rates whereas $L_1 \rightarrow H$ plants on transfer from lowered to high irradiance showed 273% increase (Figure 3.3a and Table 3.2). The percentage increase in the PSI electron transport in mesophyll thylakoid membranes of $L_1 \rightarrow H$ and $L_2 \rightarrow H$ plants to high irradiance did not correspond with the percentage decrease in the same, in $H \rightarrow L_1$ and $H \rightarrow L_2$ plants (Table 3.2). The $H \rightarrow L_2$ plants on transfer to reduced irradiance showed 75% decrease in the PSI electron transport and 306% increase in the electron transport was observed when $L_2 \rightarrow H$ plants were transferred to high light intensities in the mesophyll thylakoids of *Amaranthus* (Figure 3.3b). When $H \rightarrow L_1$ plants were transferred to reduced irradiance PSI electron transport of bundle sheath thylakoids decreased by 44% of the rates seen in H plants (Figure 3.4a) and $L_1 \rightarrow H$ plants on transfer to high irradiance showed 165% increased rates in a time period of six days. The results of transfer of $H \rightarrow L_2$ and $L_2 \rightarrow H$ plants to low light and high light regimes respectively are given in figure 3.4b. $H \rightarrow L_2$ plants showed 63% decrease in the electron transport

Figure 3.2a Effect of light intensity on whole chain electron transport rates in the bundle sheath thylakoid membranes of *Amaranthus hypochondriacus* L.

0—0 H

▲—▲ L_1

◖—◖ $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 3.2b Effect of light intensity on whole chain electron transport rates in the bundle sheath thylakoid membranes of *Amaranthus hypochondriacus* L.

0—0 H

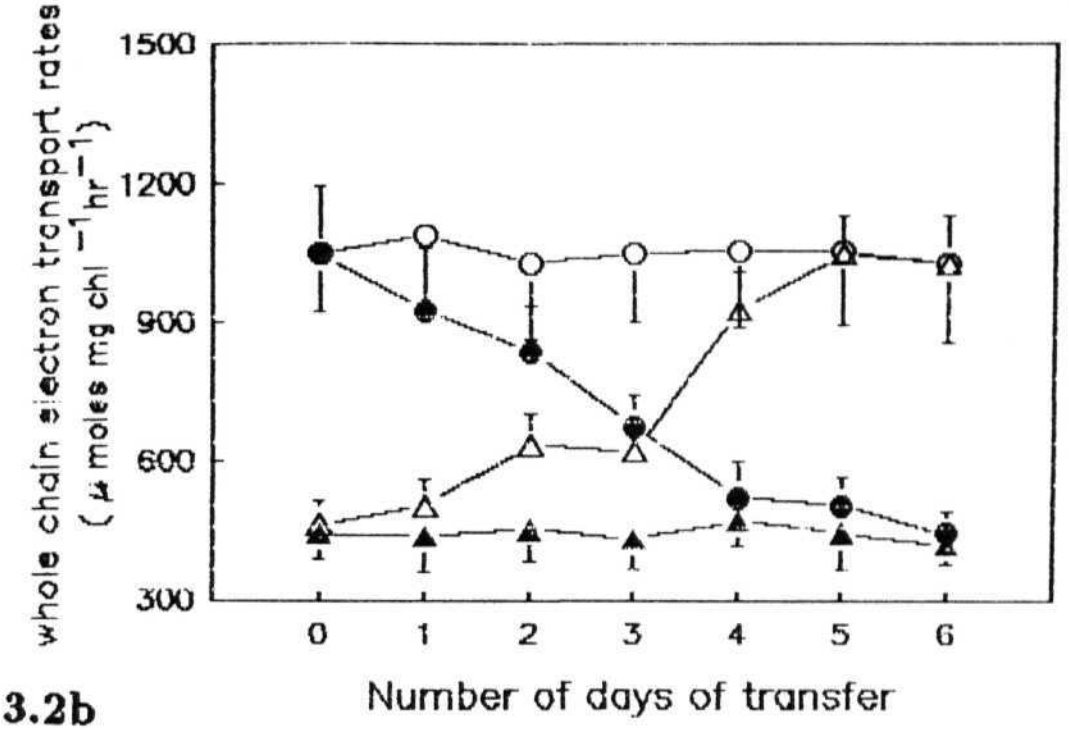
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$

The results are average of three independent experiments.

3.2a


whole chain electron transport rates
(μ moles mg chl $^{-1}$hr^{-1})
1500
1200
900
600
300
0
1
2
3
4
5
6
Number of days of transfer

3.2b

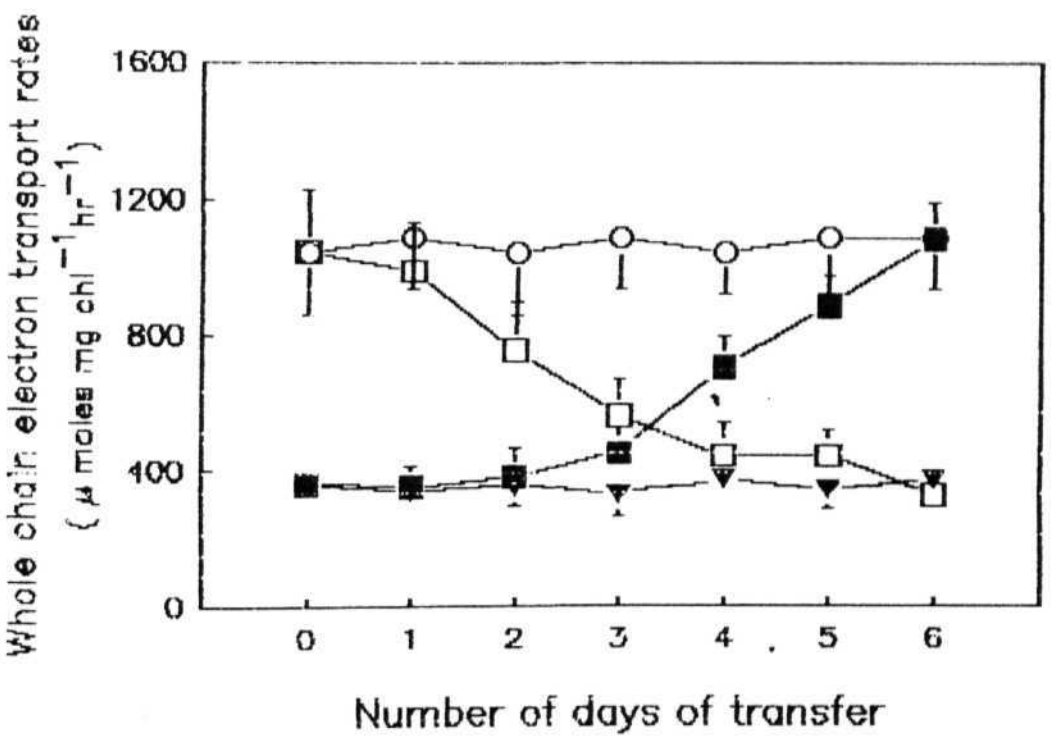
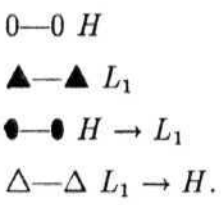
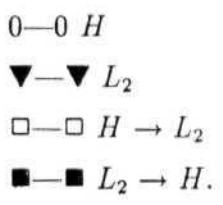

Whole chain electron transport rates
(μ moles mg chl $^{-1}$hr^{-1})
1600
1200
800
400
0
0
1
2
3
4
5
6
Number of days of transfer

Figure 3.3a. Effect of light intensity on PSI electron transport rates in the mesophyll thylakoid membranes of *Amaranthus hypochondriacus* L.



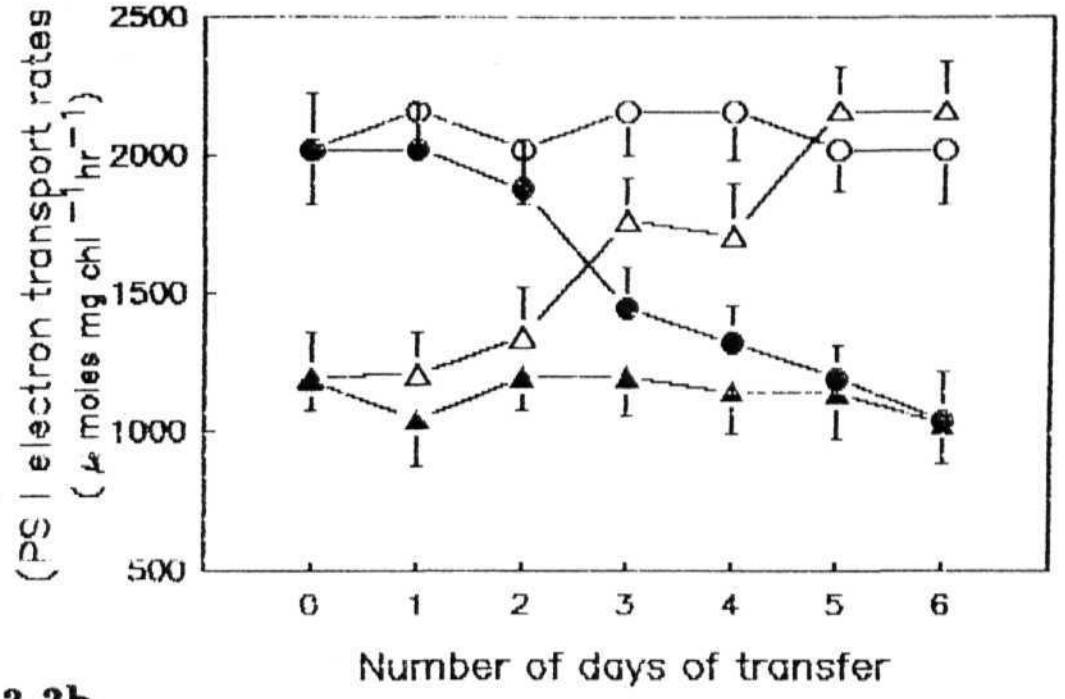
The results are average of three independent experiments.

Figure 3.3b. Effect of light intensity on PSI electron transport rates in the mesophyll thylakoid membranes of *Amaranthus hypochondriacus* L.

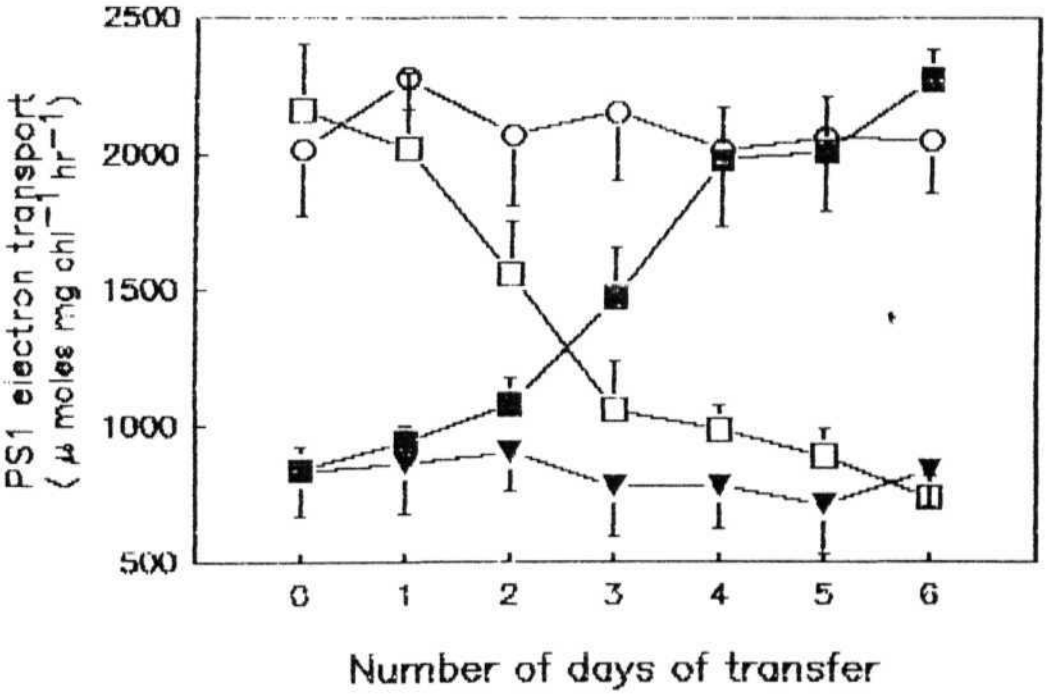


The results are average of three independent experiments.

3.3a


(PS I electron transport rates
(μ moles mg chl $^{-1}$ hr $^{-1}$)
2500
2000
1500
1000
500
0
1
2
3
4
5
6
Number of days of transfer

3.3b


PS1 electron transport
(μ moles mg chl $^{-1}$ hr $^{-1}$)
2500
2000
1500
1000
500
0
1
2
3
4
5
6
Number of days of transfer

in the bundle sheath and $L_2 \rightarrow H$ plants showed 198% increased PSI electron transport, when they were transferred to high light intensities (Figure 3.4b and Table 3.2). The reduction in the PSI electron transport in the bundle sheath was lesser compared to that in mesophyll (Table 3.2). Likewise the increase was higher on transfer to high light in mesophyll compared to bundle sheath in *Amaranthus* (Table 3.2).

PSII electron transport decreased by 67% of H when $// \rightarrow L_1$ plants were transferred to limiting light and increased by 205% in $L_1 \rightarrow //$ plants in the mesophyll of *Amaranthus*, in a span of six days (Figure 3.5a and Table 3.3). The $L_2 \rightarrow H$ plants exhibited 600% increase while $H \rightarrow L_2$ showed 86% decreased electron transport (Table 3.3 and Figure 3.5b). The increase in PSII electron transport in $L_2 \rightarrow H$ plants was gradual for 3 days and faster for next three days (Figure 3.5b). The $H \rightarrow L_1$ plants showed 62% decrease in the PSII electron transport in bundle sheath while $L_1 \rightarrow H$ plants showed 164% increase in the same (Figure 3.6a and Table 3.3). When $H \rightarrow L_2$ plants were transferred to lowered irradiance, PSII electron transport of bundle sheath thylakoids decreased by 81% to exhibit the transport observed for L_2 plants while $L_2 \rightarrow H$ plants showed 456% increase (Figure 3.6b Table 3.3). The variation of change in PSI and PSII electron transport of mesophyll was higher compared to that of bundle sheath in response to alterations in growth irradiances. (Tables 3.2 and 3.3). The time taken for changes in both mesophyll and bundle sheath thylakoids to new altered light regimes was six days.

In $H \rightarrow L\backslash$ and $H \rightarrow L_2$ *Eleusine* plants, the whole chain electron transport decreased by 57% and 72% respectively compared to *H* (Table 3.1). The reduction in the whole chain electron transport in $H \rightarrow L_1$ and $H \rightarrow L_2$ plants was observed in four and eight days respectively. (Figures 3.7a and 3.7b). In $L_1 \rightarrow H$ and $L_2 \rightarrow H$ *Eleusine* plants the electron transport increased by 130% and 256% of low light controls in four and eight

Figure 3.4a. Light intensity effect on PSI electron transport rates in the bundle sheath thylakoid membranes of *Amaranthus hypochondriacus.* L.

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 3.4b. Light intensity effect on electron transport rates in bundle sheath thylakoid membranes of *Amaranthus hypochondriacus* L.

0—0 H

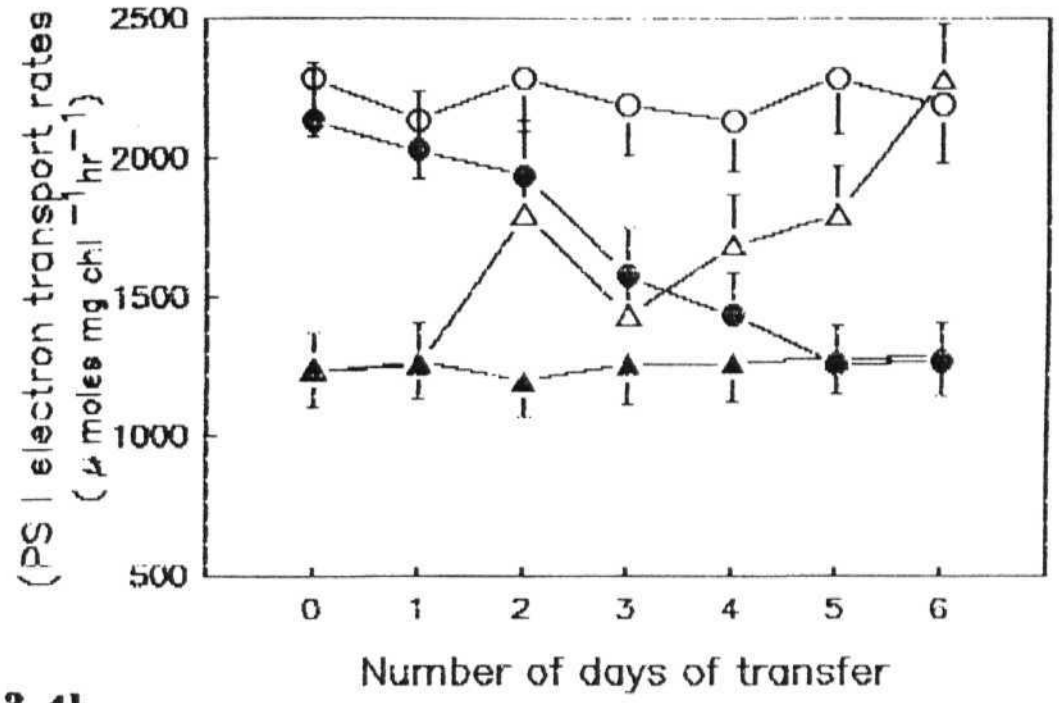
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments

3.4a


(PS I electron transport rates
(μ moles mg chl $^{-1}$ hr $^{-1}$)
2500
2000
1500
1000
500
0
1
2
3
4
5
6
Number of days of transfer

3.4b

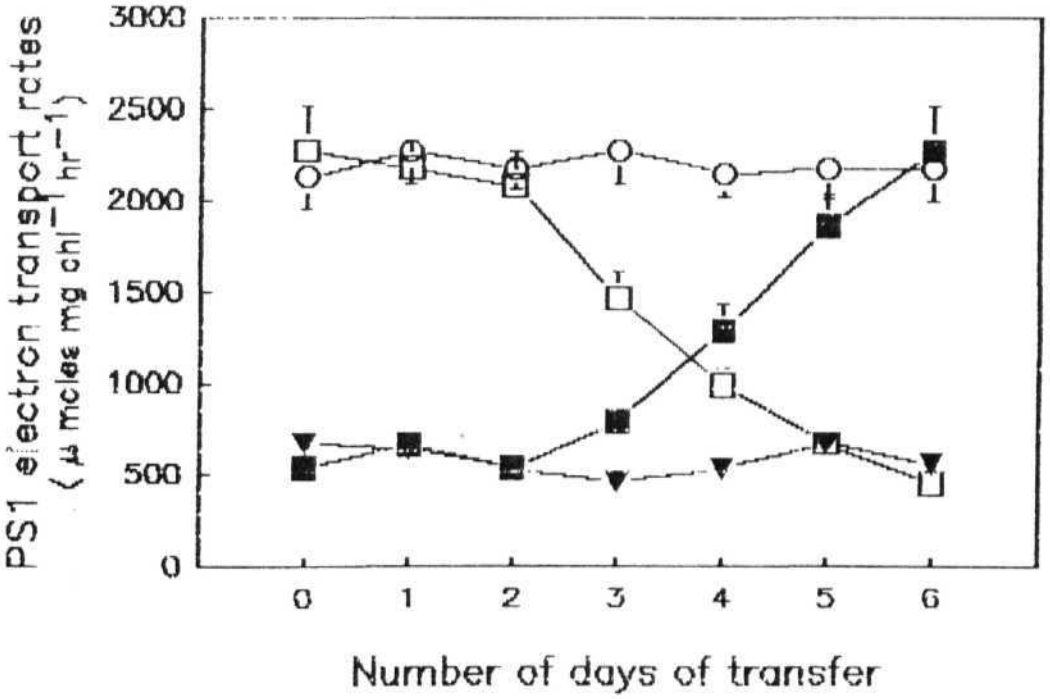
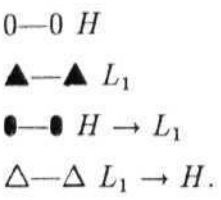
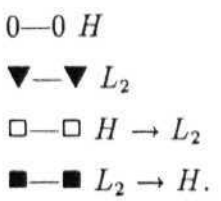

PS1 electron transport rates
(μ moles mg chl $^{-1}$ hr $^{-1}$)
3000
2500
2000
1500
1000
500
0
0
1
2
3
4
5
6
Number of days of transfer

Figure 3.5a. Effect of light intensity on PSII electron transport rates in the mesophyll thylakoid membranes of *Amaranthus hypochondriacus* L.



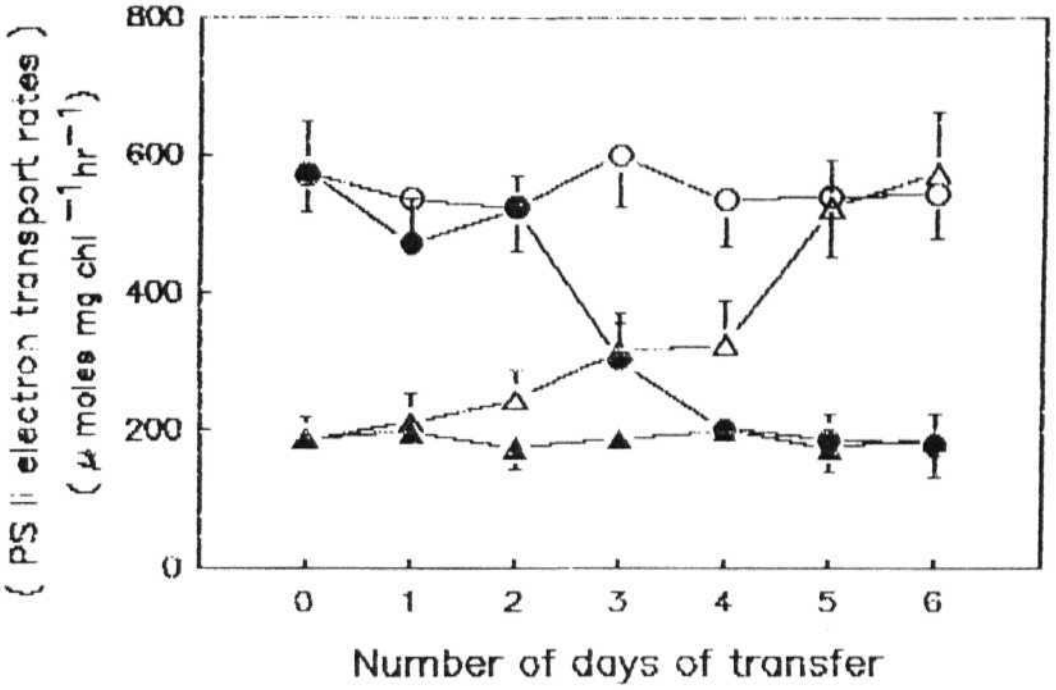
The results are average of three independent experiments.

Figure 3.5b. Light intensity effect on PSII electron transport rates in the mesophyll thylakoid membranes of *Amaranthus hypochondriacus* L.



The results are average of three independent experiments.

3.5a


(PS II electron transport rates)
(μ moles mg chl $^{-1}$hr $^{-1}$)
800
600
400
200
0
0 1 2 3 4 5 6
Number of days of transfer

3.5b

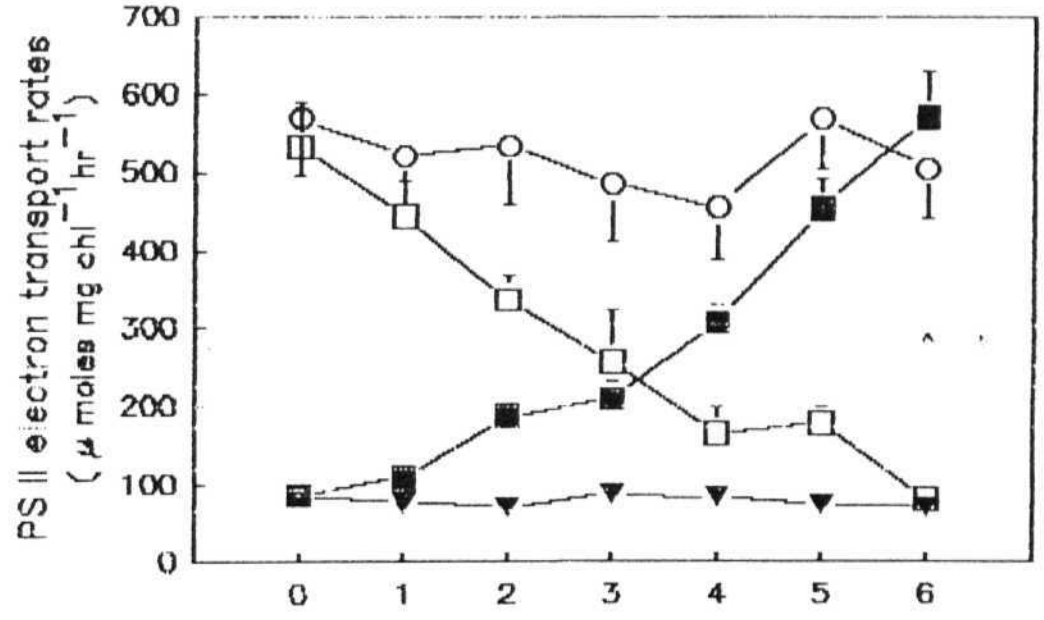
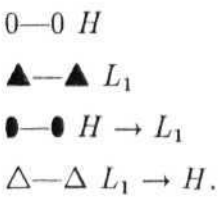
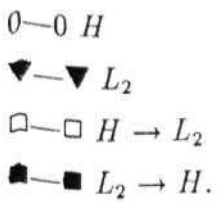

PS II electron transport rates
(μ moles mg chl $^{-1}$hr $^{-1}$)
700
600
500
400
300
200
100
0
0 1 2 3 4 5 6
Number of days of transfer

Figure 3.6a. PSII electron transport rates in bundle sheath thylakoid membranes of *Amaranthus hypochondriacus* L. under various light intensities.



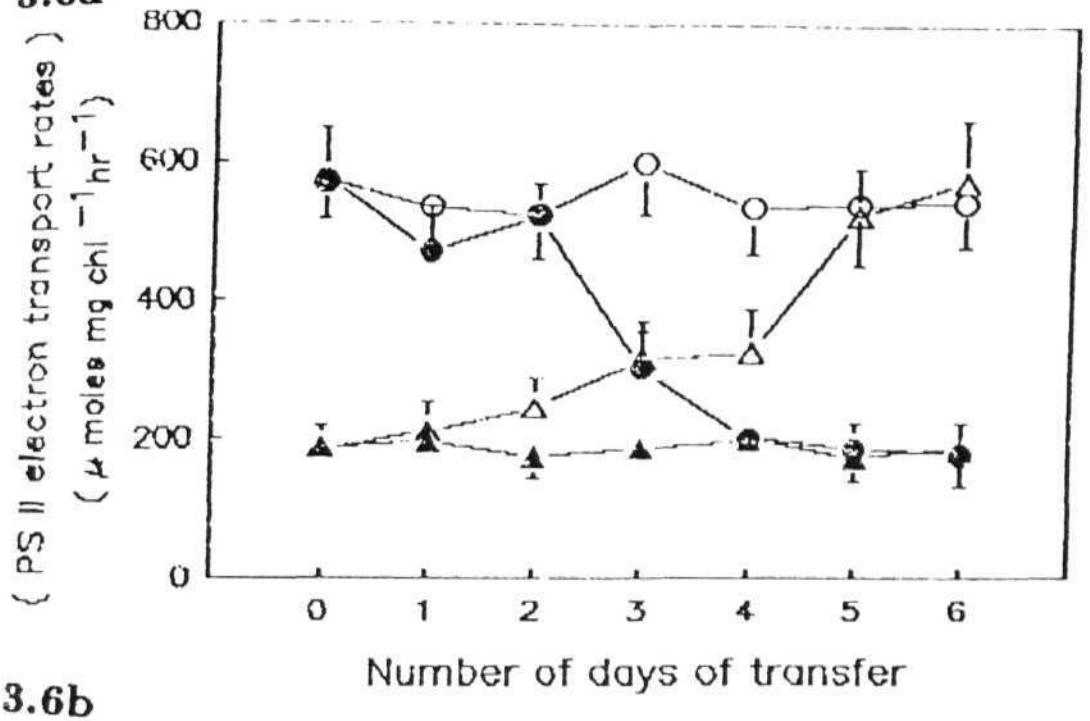
The results are average of three independent experiments.

Figure 3.6b. PSII electron transport rates in bundle sheath thylakoid membranes of *Amaranthus hypochondriacus* L. under various light intensities.



The results are average of three independent experiments.

3.6a


(PS II electron transport rates)
(μ moles mg chl $^{-1}$ hr $^{-1}$)
800
600
400
200
0
0 1 2 3 4 5 6
Number of days of transfer

3.6b

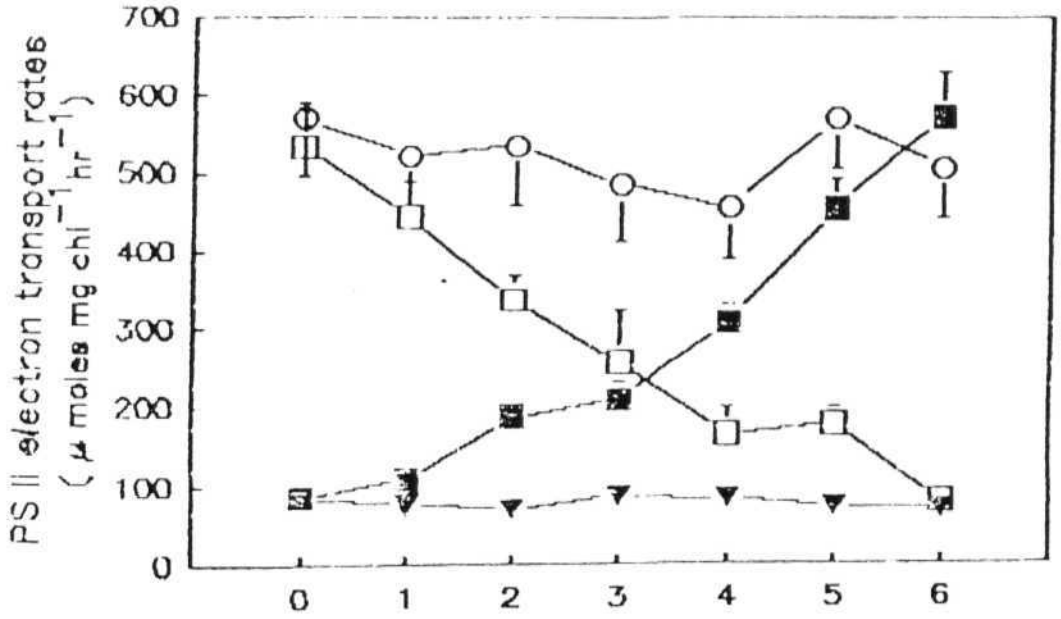
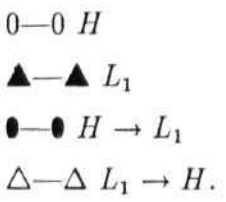
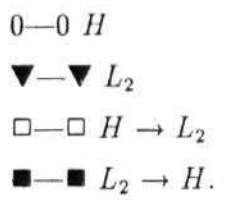

PS II electron transport rates
(μ moles mg chl $^{-1}$ hr $^{-1}$)
700
600
500
400
300
200
100
0
0 1 2 3 4 5 6
Number of days of transfer

Figure 3.7a. Light intensity effect on whole chain electron transport rates in thylakoid membranes of *Eleusine coracana.*



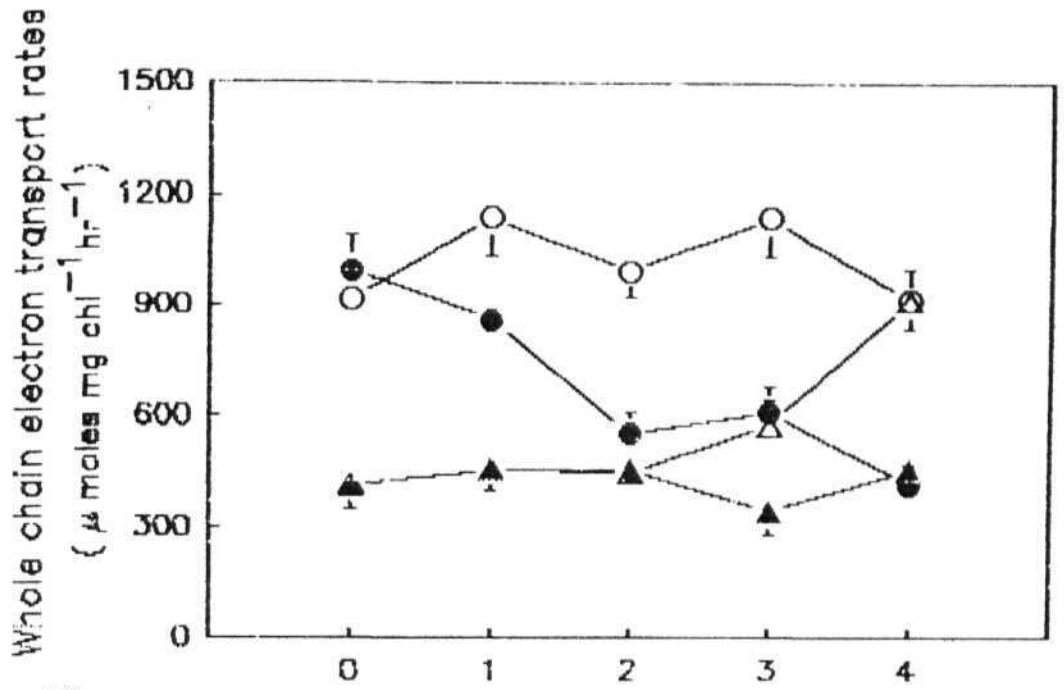
The results are average of three independent experiments.

Figure 3.7b. Light intensity effect on wliole chain electron transport rates in thylakoid membranes of *Eleusine coracana.*

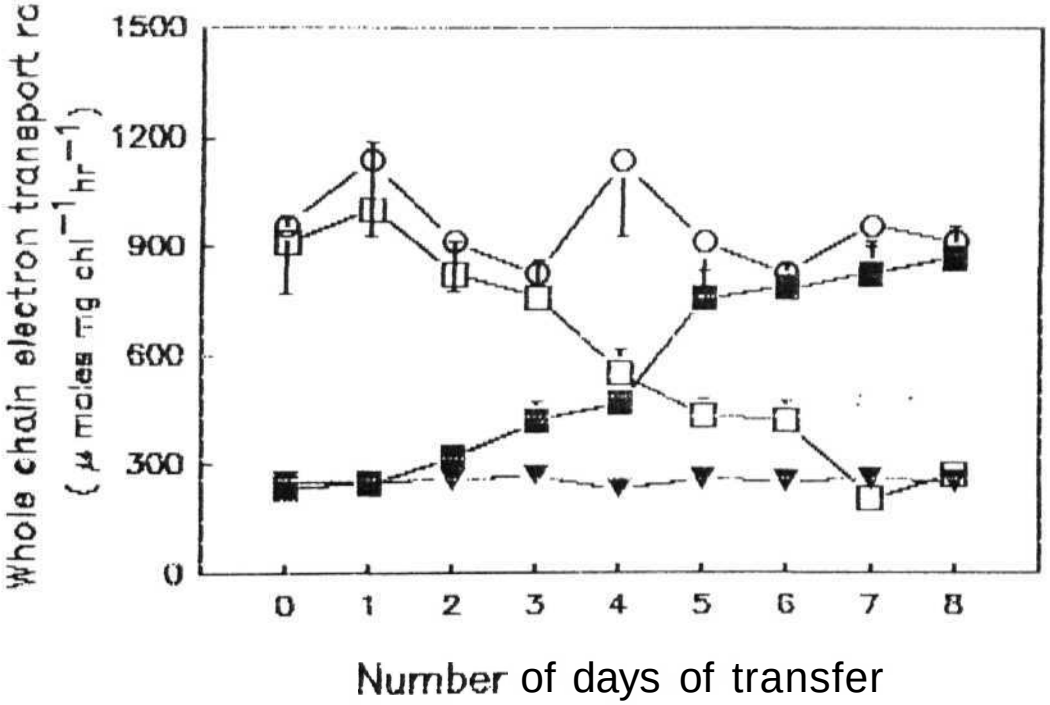


The results are average of three independent experiments.

3.7a


Whole chain electron transport rates
(μ moles mg chl^{-1}hr^{-1})
1500
1200
900
600
300
0
0
1
2
3
4
Number of days of transfer

3.7b


Whole chain electron transport rates
(μ moles mg chl^{-1}hr^{-1})
1500
1200
900
600
300
0
0
1
2
3
4
5
6
7
8
Number of days of transfer

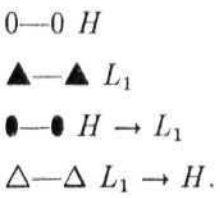
days respectively with an initial lag of forty eight hours (Tables 3.1; Figures 3.7a and 3.7b).

The PSI electron transport of $H \rightarrow L_1$ and $H \rightsquigarrow L_2$ plants of *Eleusine* decreased by 63% and 77% of *H* plants and approached the rates of L_1 and L_2 in four and eight days respectively (Figures 3.8a and 3.8b; Table 3.2). There was a lag of twenty four hours observed before the effect of reduced irradiances were manifested. (Figures 3.8a and 3.8b). PSI electron transport rates increased in $L_1 \rightarrow$ *11* and $L_2 \rightarrow$ // plants of *Eleusine* by 256% and 343% respectively with initial lag of forty eight hours (Figures 3.8a and 3.8b, Table 3.2).

In $H \rightarrow L_1$ and $H \rightarrow L_2$ plants of *Eleusine*, PSII electron transport decreased by 71% and 86% in four and eight days respectively, (Figures 3.9a and 3.9b and Table 3.3) to obtain the transport observed in L_1 and L_2 plants. The $L_1 \rightarrow$ // and $L_2 \rightarrow H$ *Eleusine* plants showed increase in the PSII electron transport by 247% and 660% in a. time period of four and eight days respectively (Figures 3.9a and 3.9b; Table3.3). PSII electron transport was more sensitive to changes in irradiances in contrast to PSI electron transport in *Eleusine* (Tables 3.2 and 3.3).

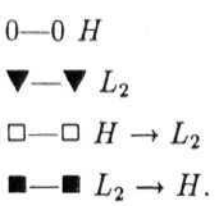
The whole chain electron transport in thylakoid membranes of // $\rightarrow L_1$ and $H \rightarrow L_2$ plants of *Gomphrena* decreased by 54% and 65% respectively (Table 3.1; Figures 3.10a and 3.10b). The electron transport increased by 118% and 184% in $L_1 \rightarrow$ *11* and $L_2 \rightarrow H$ plants after an initial lag, in eight and ten days respectively (Figures 3.10a and 3.10b; Table 3.1). The $H \rightarrow L_1$ and $H \rightarrow L_2$ *Gomphrena* plants showed 52% and 73% reduction in PSI electron transport while the electron transport increased (184% and 234% of L_1 and L_2) in $L\backslash \rightarrow H$ and $L_2 \rightarrow$ // plants (Figures 3.11a and 3.11b; Table 3.2).

Figure 3.8a. PSI electron transport rates in thylakoid membranes of *Eleusine coracana* under varying light.



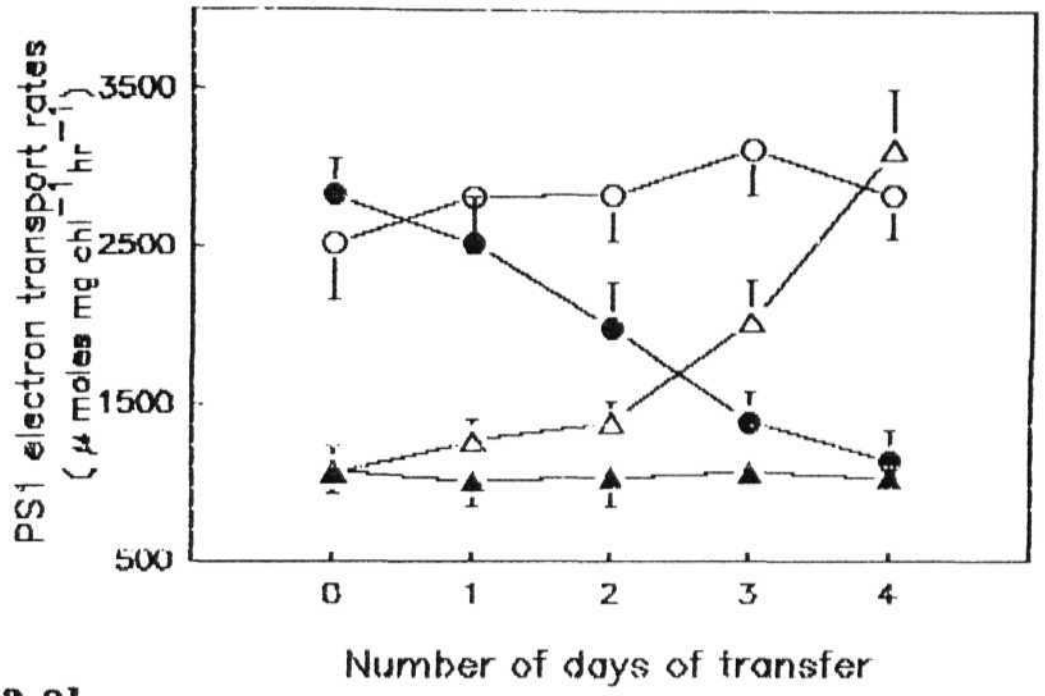
The results are average of three independent experiments.

Figure 3.8b. PSI electron transport rates in thylakoid membranes of *Eleusine coracana* under varying light



The results are average of three independent experiments.

3.8a


PS1 electron transport rates
(μ moles mg chl^{-1} hr^{-1})
3500
2500
1500
500
0
1
2
3
4
Number of days of transfer

3.8b

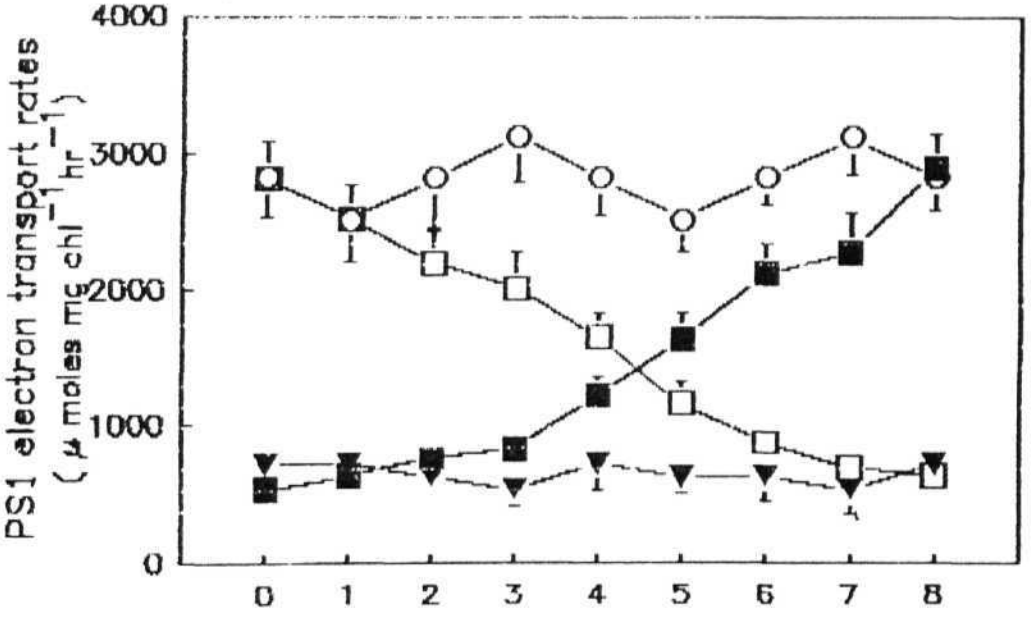

PS1 electron transport rates
(μ moles mg chl^{-1} hr^{-1})
4000
3000
2000
1000
0
0
1
2
3
4
5
6
7
8
Number of days of transfer

Figure 3.9a. Effect of light intensity on PSII electron transport rates in thylakoid membranes of *Eleusine coracana*

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 3.9b. Effect of light intensity on PSII electron transport rates in thylakoid membranes of *Eleusine coracana* •

0—0 H

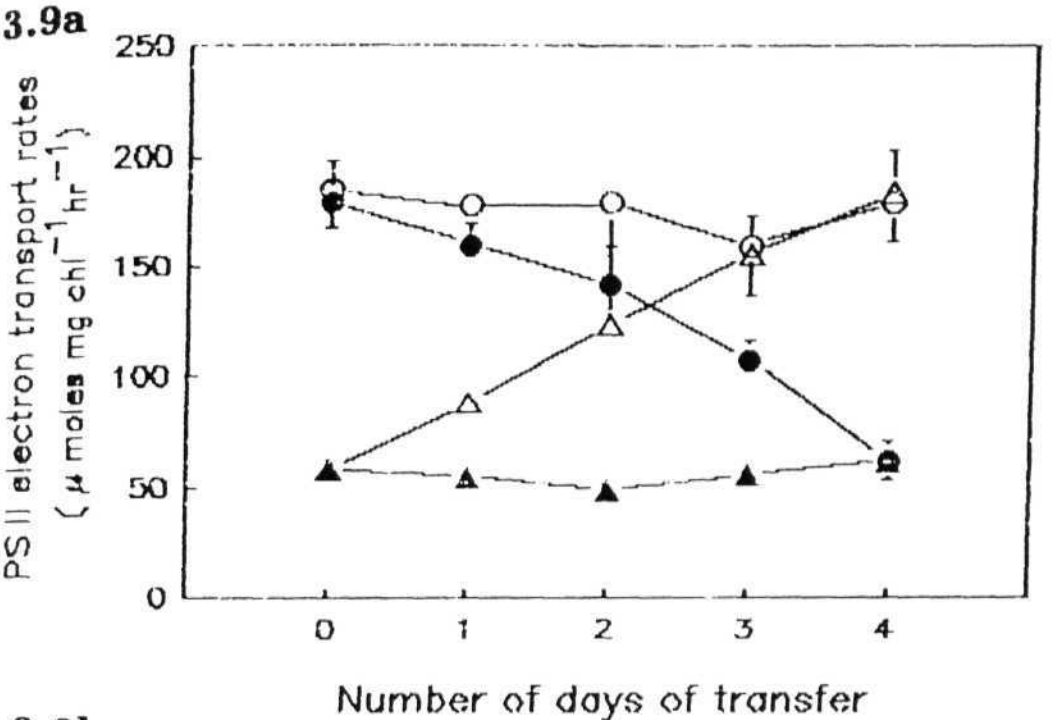
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

3.9a


PS II electron transport rates
(μ moles mg chl^{-1} hr^{-1})
250
200
150
100
50
0
0
1
2
3
4
Number of days of transfer

3.9b

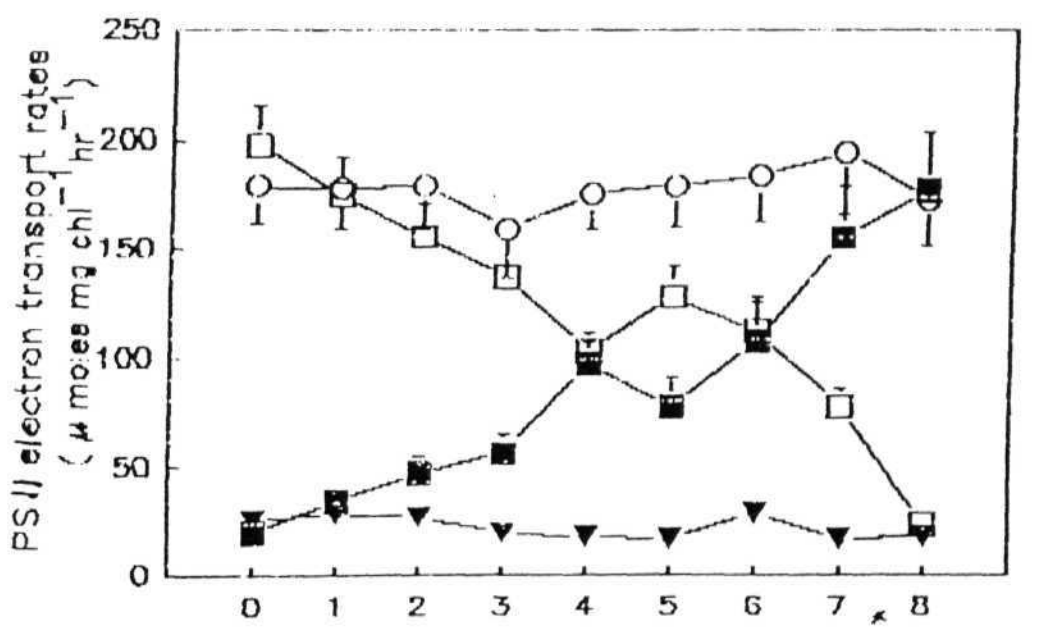

PS II electron transport rates
(μ moles mg chl^{-1} hr^{-1})
250
200
150
100
50
0
0
1
2
3
4
5
6
7
8
Number of days of transfer

Figure 3.10a. Light intensity effect on whole chain electron transport rates in thylakoid membranes of *Gomphrena globosa* .

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 3.10b. Light intensity effect on whole chain electron transport rates in thylakoid membranes of *Gomphrena globosa* .

0—0 H

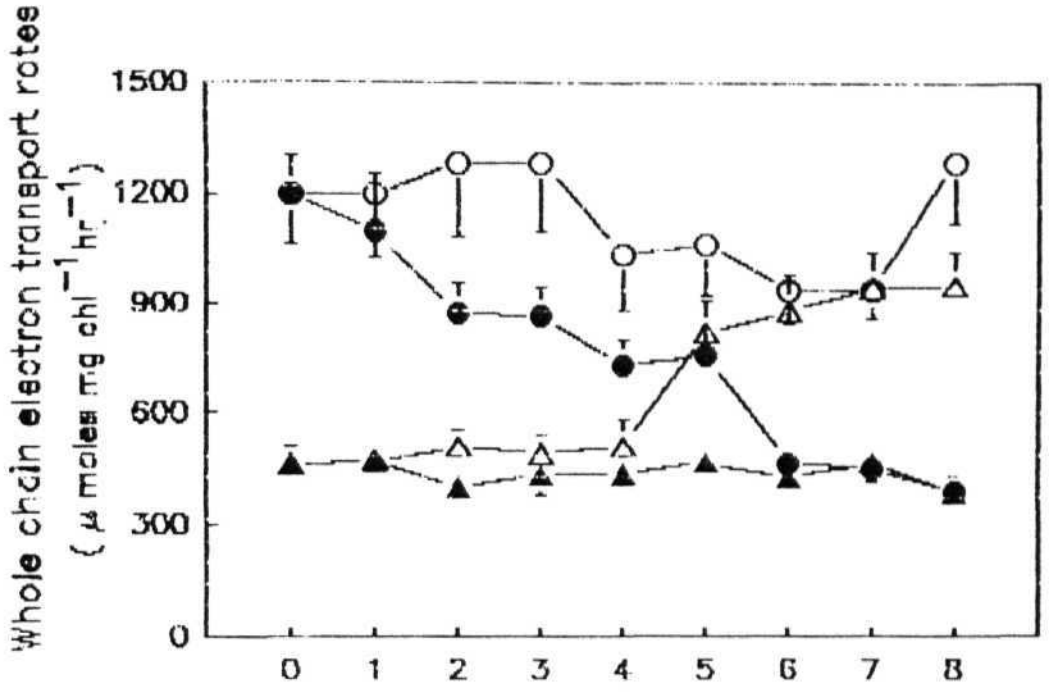
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

3.10a


Whole chain electron transport rates
(μ moles mg chl^{-1} hr^{-1})
1500
1200
900
600
300
0
0 1 2 3 4 5 6 7 8
Number of days of transfer

3.10b

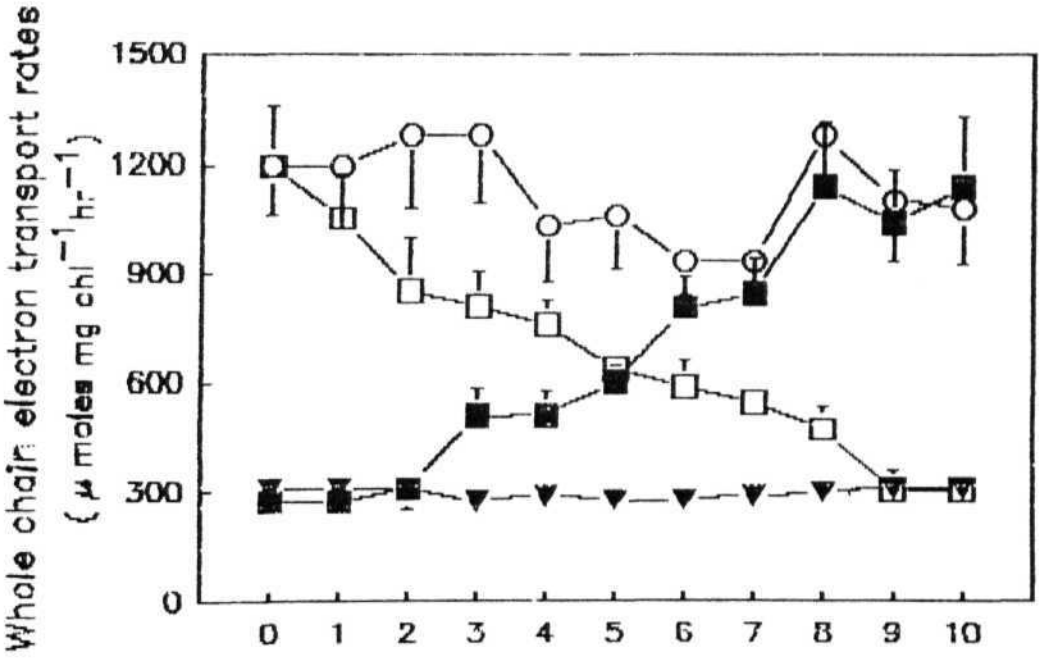

Whole chain electron transport rates
(μ moles mg chl^{-1} hr^{-1})
1500
1200
900
600
300
0
0 1 2 3 4 5 6 7 8 9 10
Number of days of transfer

Figure 3.11a. Effect of light intensity on PSI electron transport rates in thylakoid membranes of *Gomphrena globosa* •

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 3.11b. Effect of light intensity on PSI electron transport rates in thylakoid membranes of *Gomphrena globosa*.

0—0 H

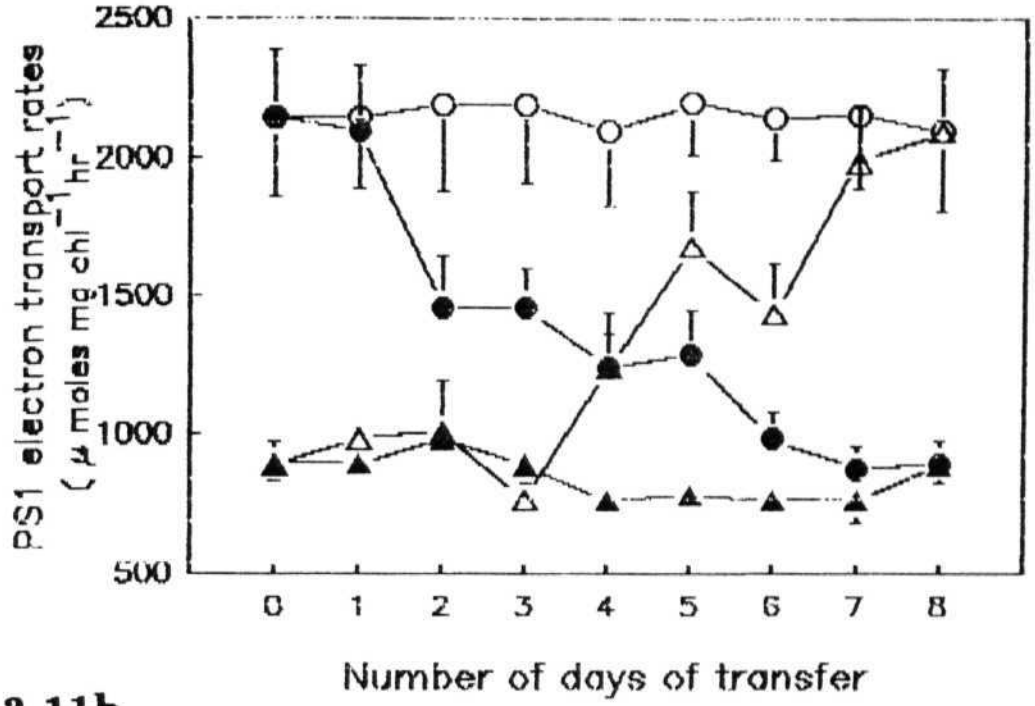
▼—▼ L_2

□—□ $H \rightarrow L_2$

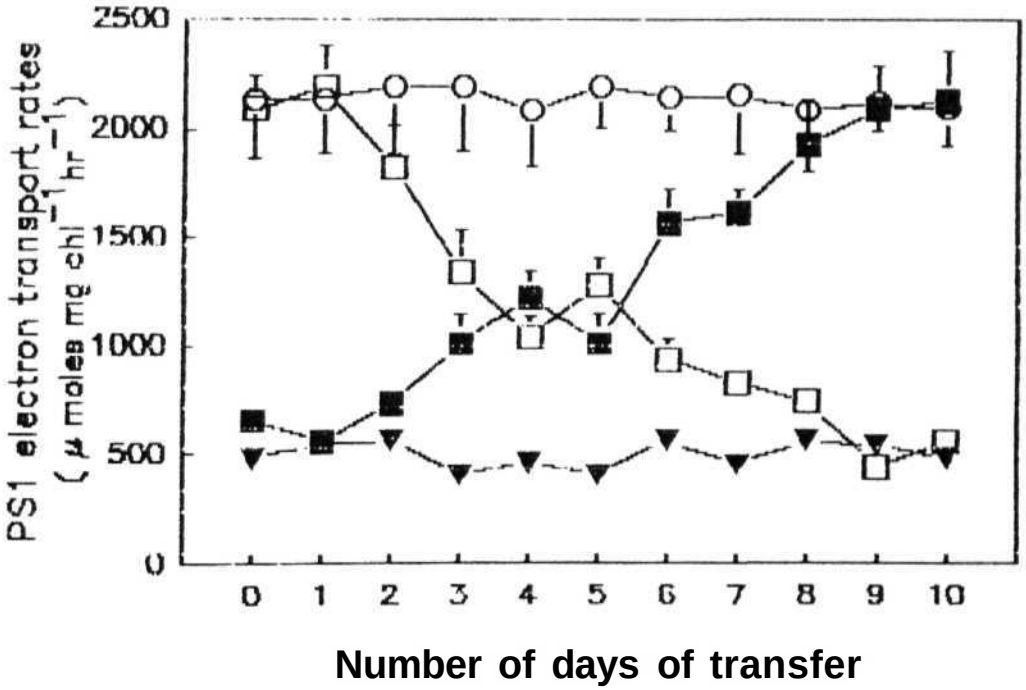
■—■ $L_2 \rightarrow H$.

The results arc average of three independent experiments.

3.11a


PS1 electron transport rates
(μ moles mg chl^{-1} hr^{-1})
2500
2000
1500
1000
500
0 1 2 3 4 5 6 7 8
Number of days of transfer

3.11b


PS1 electron transport rates
(μ moles mg chl^{-1} hr^{-1})
2500
2000
1500
1000
500
0
0 1 2 3 4 5 6 7 8 9 10
Number of days of transfer

In $H \rightarrow L_1$ and $H \rightarrow L_2$ plants of *Gomphrena,* PSII electron transport decreased by 61% and 81% of the transport observed for *H* plants (Table 3.3; Figures 3.12a and 3.12b). The PSII electron transport increased by 160% and 439% in $L_1 \rightarrow H$ and $L_2 \rightarrow H$ plants (Table 3.3; Figures 3.12a and 3.12b). The time taken to manifest the observed modulation of whole chain, PSI and PSII electron transport was eight and ten days, to one third and one tenth of normal irradiances. A lag of forty eight hours was observed prior to the noticeable variation in electron transport in response to changes in growth light intensities. PSII electron transport was more susceptible to changes in the irradiance compared to PSI electron transport in *Gomphrena* (Tables 3.2 and 3.3).

A least alteration in whole chain, PSI and PSII electron transport was observed in *Gomphrena,* in response to variation in light intensities, compared to that in *Amaranthus* and *Eleusine.* The comparison of percentage change in electron transport, due to variation in light intensities, between the species studied here was more significant statistically, when calculated with respect to low light controls. Therefore the observed % decrease on transfer of plants from high to lowered light was considered noteworthy when comparison was made between the species.

Components of thylakoid membrane

In $L\backslash$ and L_2 plants of *Amaranthus,* P_{700} content decreased by 38% and 56% in mesophyll (Figures 3.13a and 3.13b) and 13.2% and 25% in bundle sheath thylakoid membranes respectively. (Figures 3.14a and 3.14b). The $H \rightarrow L\backslash$ and $H \rightarrow L_2$ plants registered 38% and 56% decrease in the P_{700} content in mesophyll whereas 13.2% and

Figure 3.12a. Light intensity effect on PSII election transport rates in thylakoid membranes of *Gomphrena globosa*.

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 3.12b. Light intensity effect on PSII electron transport rates in thylakoid membranes of *Gomphrena globosa*.

0—0 H

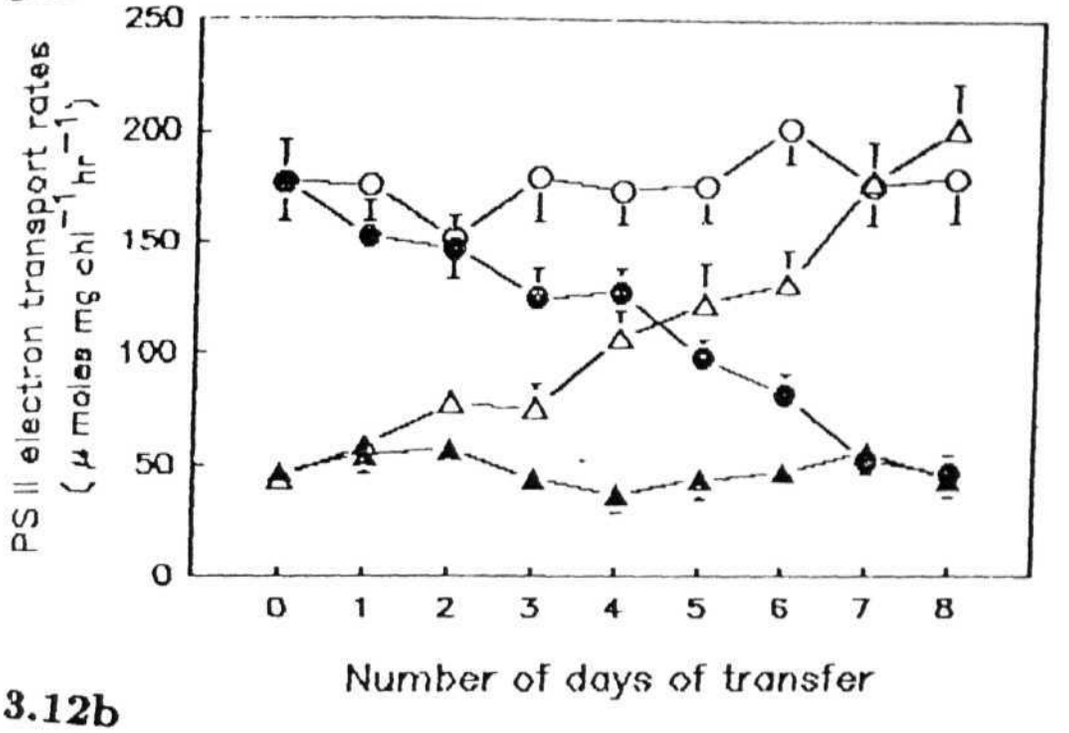
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

3.12a


PS II electron transport rates
(μ moles mg chl^{-1} hr^{-1})
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8
Number of days of transfer

3.12b

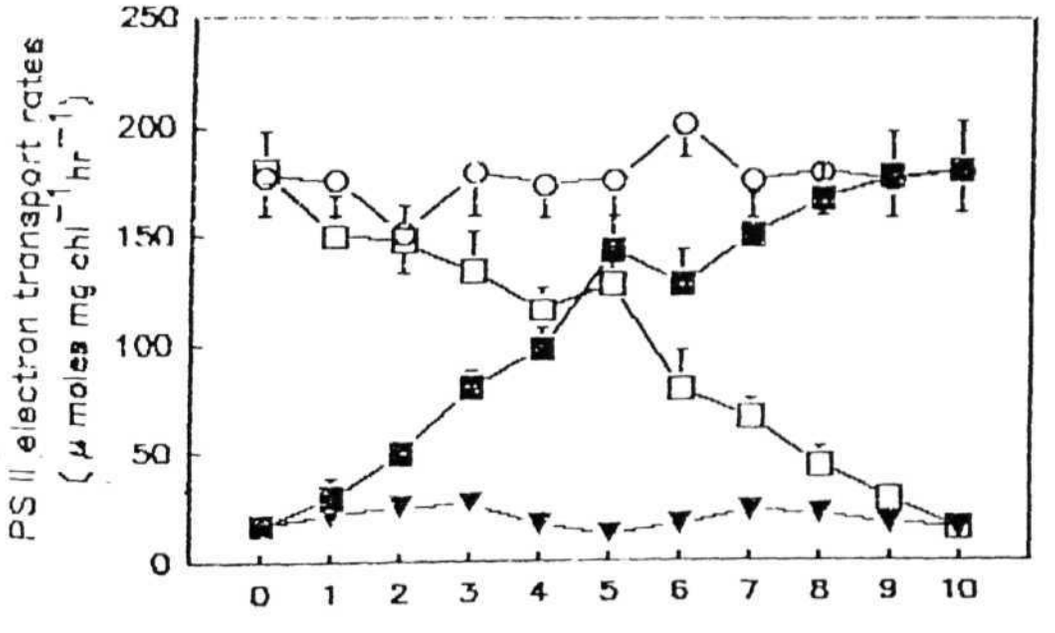
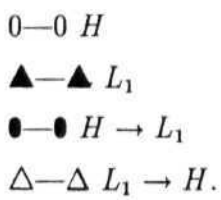
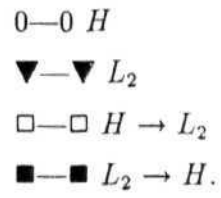

PS II electron transport rates
(μ moles mg chl^{-1} hr^{-1})
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10
Number of days of transfer

Figure 3.13a. Light intensity effect on P_{700} content in mesophyll thylakoid membranes of *Amaranthus hypochondriacus* L.



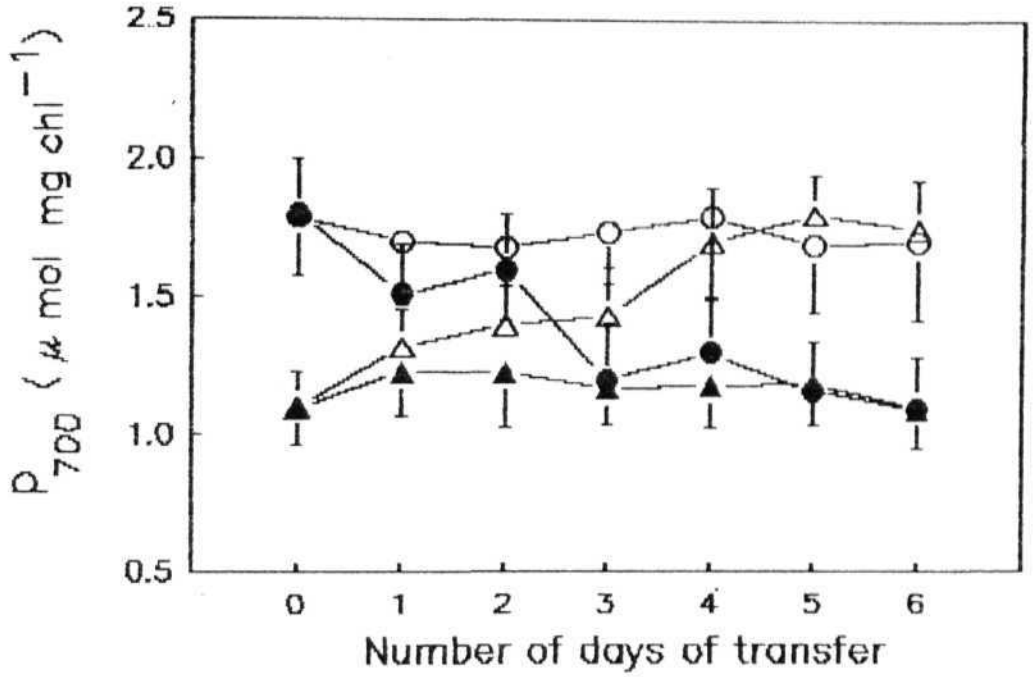
The results are average of three independent experiments.

Figure 3.13b. Light intensity effect on P_{700} content in mesophyll thylakoid membranes of *Amaranthus hypochondriacus* L.

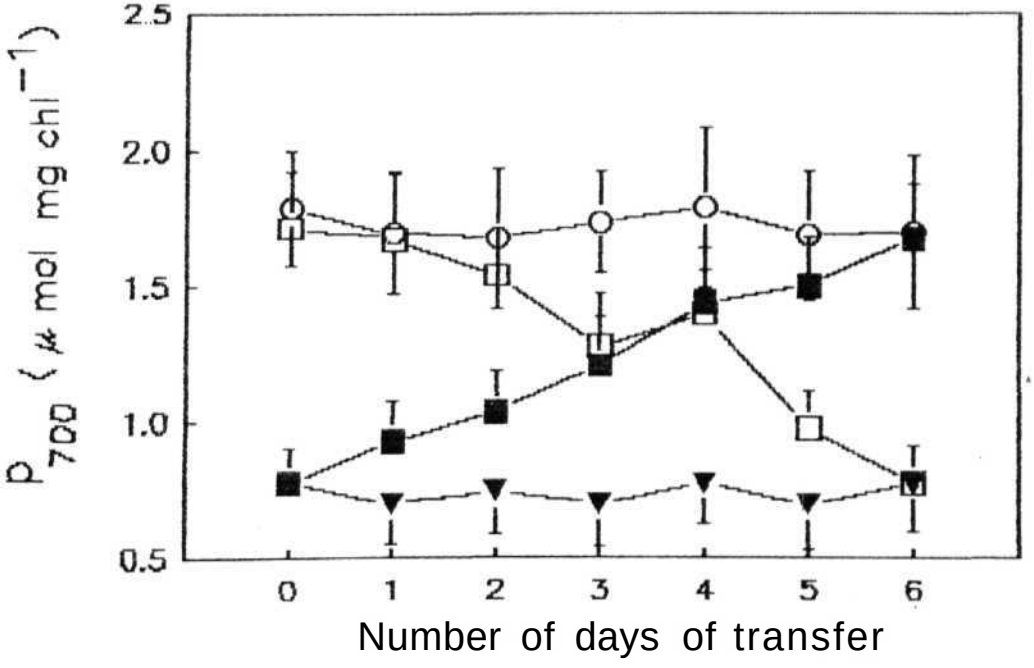


The results are average of three independent experiments.

3.13a


2.5
2.0
1.5
1.0
0.5
P_{700} (μ mol mg chl^{-1})
0
1
2
3
4
5
6
Number of days of transfer

3.13b

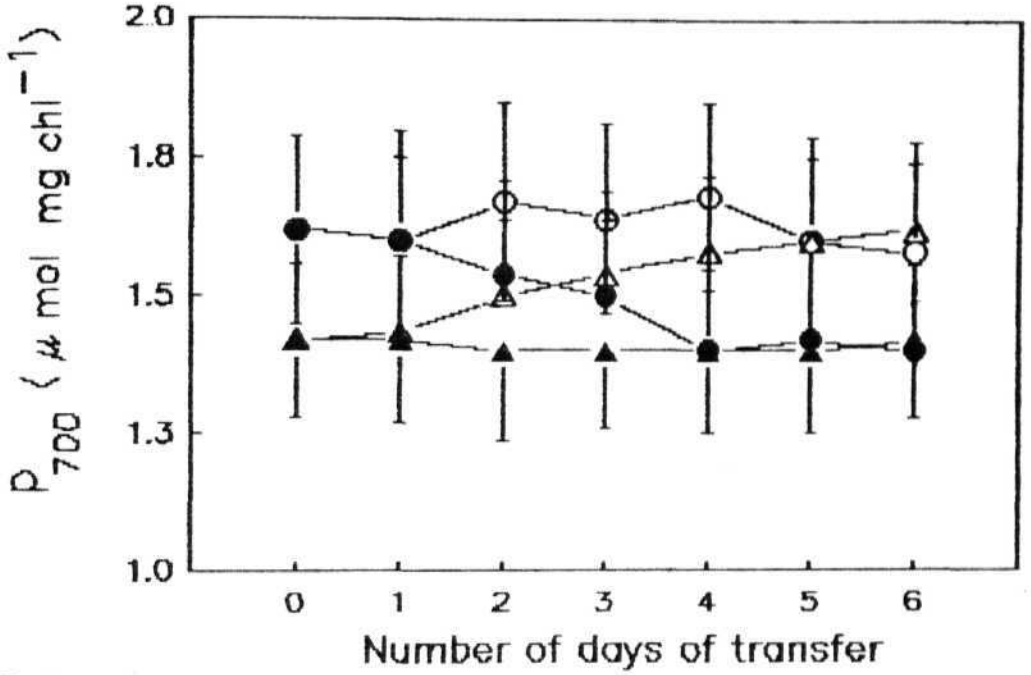

2.5
2.0
1.5
1.0
0.5
P_{700} (μ mol mg chl^{-1})
0
1
2
3
4
5
6
Number of days of transfer

25% decrease was observed in bundle sheath thylakoid membranes respectively in a span of six days. However, in $L_1 \rightarrow H$ and $L_2 \rightarrow H$ plants, P_{700} content increased by 63% and 126% of the L_1 and L_2 values in mesophyll and 16% and 35% in bundle sheath thylakoids respectively (Figures 3.13a, 3.13b, 3.14a and 3.14b; Table 3.4). The reduction in P_{700} content in bundle sheath thylakoids was lesser than that of mesophyll at similar lowered light intensities (Table 3.4).

The L_1 and L_2 plants of *Amaranthus* registered 39% and 55% decline in cytochrome / content in the mesophyll thylakoids, whereas, 56% and 67% decrease was observed in the bundle sheath. The $H \rightarrow L_1$ and // $\rightarrow L_2$ plants of *Amaranthus* showed 39% and 55% reduction respectively in cytochrome / content (Figures 3.15a and 3.15b). However, the bundle sheath exhibited 48% and 65% decrease compared to the rates in *H* plants (Figures 3.16a and 3.16b; Table 3.4). In $L_1 \rightarrow H$ and $L_2 \rightarrow H$ *Amaranthus* plants, the cytochrome / content in mesophyll increased by 64% and 120% (Figures 3.15a and 3.15b) while the content increased by 124% and 205%) in bundle sheath. (Figures 3.16a and 3.16b; Table 3.4). The time taken for modulation of cytochrome / to different light regimes was identical in mesophyll and bundle sheath thylakoid membranes of *Amaranthus.* The duration of six days for the adjustment of cytochrome / levels correlated with the time taken for alteration of whole chain, PSI and PSII electron transport rates in mesophyll and bundle sheath thylakoid membranes of *Amaranthus.*

Chlorophyll/cytochrome f ratio increased in mesophyll and bundle sheath thylakoid membranes of L_1 and L_2 *Amaranthus* plants respectively. The chlorophyll /cytochrome f ratio of $L\backslash \rightarrow H$ and $L_2 \rightarrow H$ plants decreased by 39% and 55% of the controls in mesophyll and 56% and 68% in bundle sheath respectively (Figures 3.17a, 3.17b, 3.18a

3.14a


2.0
1.8
1.5
1.3
1.0
P_{700} (μ mol mg chl^{-1})
0
1
2
3
4
5
6
Number of days of transfer

3.14b

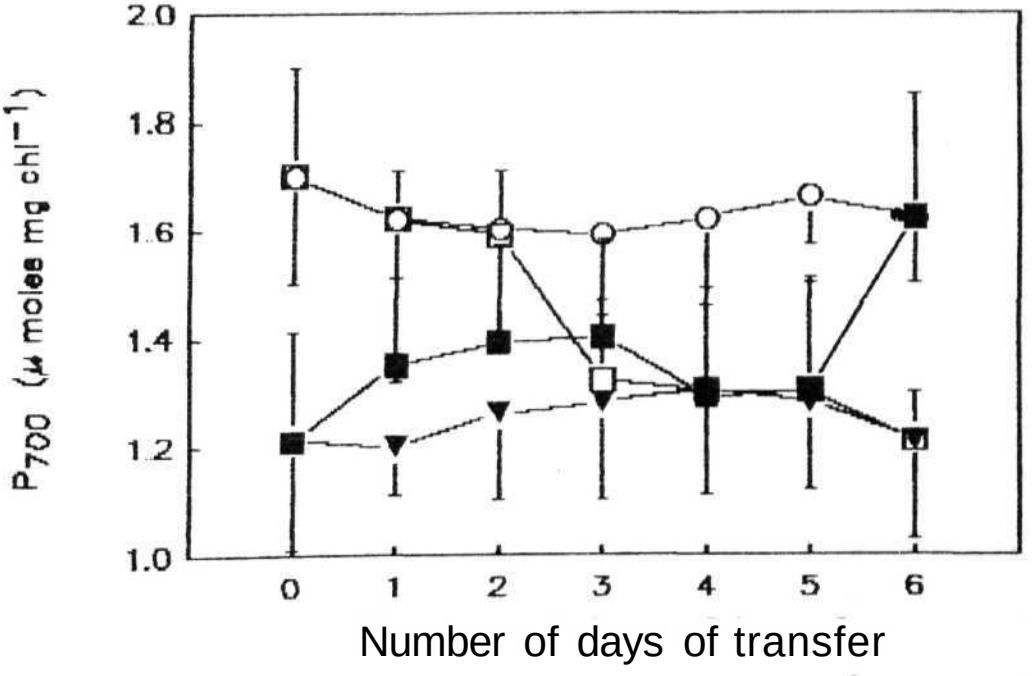

2.0
1.8
1.6
1.4
1.2
1.0
P_{700} (μ moles mg chl^{-1})
0
1
2
3
4
5
6
Number of days of transfer

Table 3.4: Effect of reduced irradiance on supra molecular complexes of thylakoids

Species	Components	Content under different light (μmoles mg chl^{-1})			% decrease of control on acclimation		%increase of control on acclimattion	
		H	L_1	L_2	$L_1 \rightarrow H$	$L_2 \rightarrow H$	$H \rightarrow L_1$	$H \rightarrow L_2$
Amaranthus Mesophyll	P_{700}	1.791±0.20	1.10±0.09[a]	0.79±0.08[a]	38	56	63	126
	Cyt f**	3.300±0.05	2.01±0.34[a]	1.50±0.21[a]	39	55	64	120
	$\frac{\text{Cyt f}}{P_{700}}$**	†1.84	1.82	1.89	—	—	—	—
Bundle sheath	P_{700}	1.620±0.18	1.40±0.09[c]	1.21±0.09[a]	13	25	16	34
	Cyt f**	2.900±0.18	1.29±0.09[a]	0.95±0.09[a]	56	67	24	205
	$\frac{\text{Cyt f}}{P_{700}}$**	†1.79	0.92[a]	0.78[a]	49	56	95	129
Eleusine	Cyt f**	3.600±0.30	2.12±0.23[a]	0.78±0.08[a]	41	78	69	361
Gomphrena	Cyt f**	3.380±0.40	2.33±0.30[a]	1.36±0.16[a]	31	59	46	148

†It is a ratio with no units.

**Cytocrome f

The results are average of three independent experiments

H : Thylakoid membranes from high light (2000 μE $m^{-2}s^{-1}$) grown plants.

L_1: Thylakoid membranes from low light (650 μE $m^{-2}s^{-1}$) grown plants.

L_2: Thylakoid membranes from low light (200 μE $m^{-2}s^{-1}$) grown plants.

$L_1 \rightarrow H$: Thylakoid membranes from low light (650 μE $m^{-2}s^{-1}$) grown plants after acclimation to high irradiance (2000 μE $m^{-2}s^{-1}$).

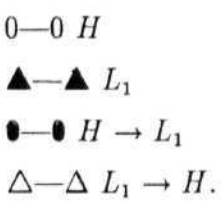
$L_2 \rightarrow H$: Thylakoid membranes from low light (200μE $m^{-2}s^{-1}$) grown plants acclimated to high irradiance (2000 μE $m^{-2}s^{-1}$).

$H \rightarrow L_1$: Thylakoid membranes from high irradiance (2000 μE $m^{-2}s^{-1}$) grown plants acclimated to reduced light (650 μE $m^{-2}s^{-1}$).

$H \rightarrow L_2$: Thylakoid membranes from high irradiance (2000 μE $m^{-2}s^{-1}$) grown plants acclimated to reduced light (200 μE $m^{-2}s^{-1}$).

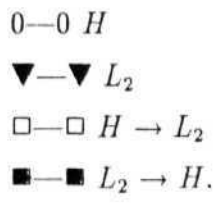
a = $p < 0.001$ c = $p < 0.02$

Figure 3.15a. Light intensity effect on cytochrome / content in mesophyll thylakoid membranes of *Amaranthus hypochondriacus* L.



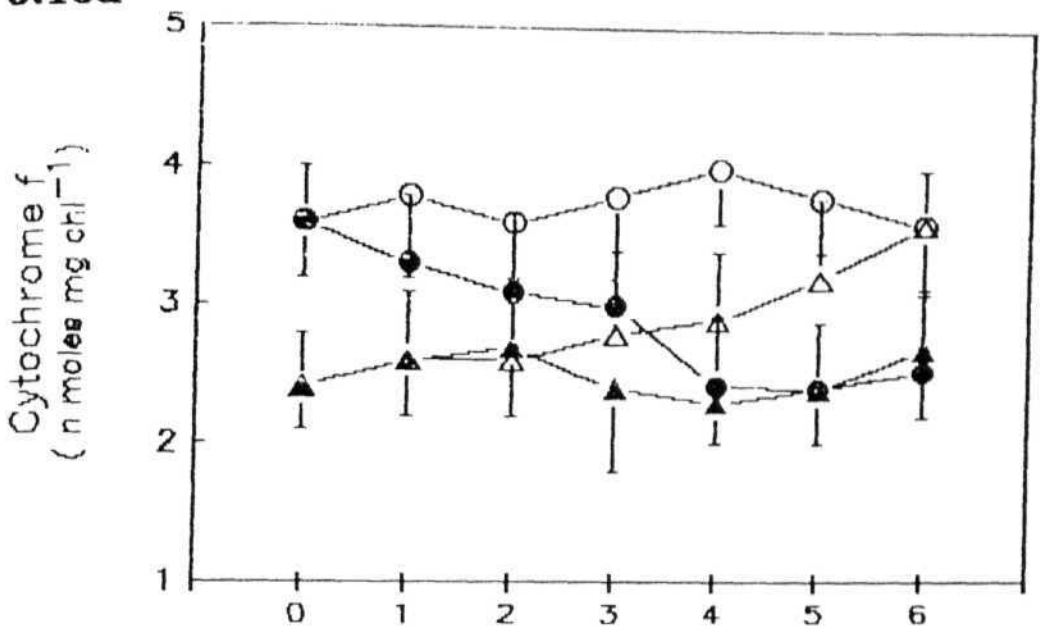
The results are average of three independent experiments.

Figure 3.15b. Light intensity effect on cytochrome / content in mesophyll thylakoid membranes of *Amaranthus hypochondriacus* L.



The results are average of three independent experiments.

3.15a


Cytochrome f
(n moles mg chl^{-1})
5
4
3
2
1
0
1
2
3
4
5
6
Number of days of transfer

3.15b

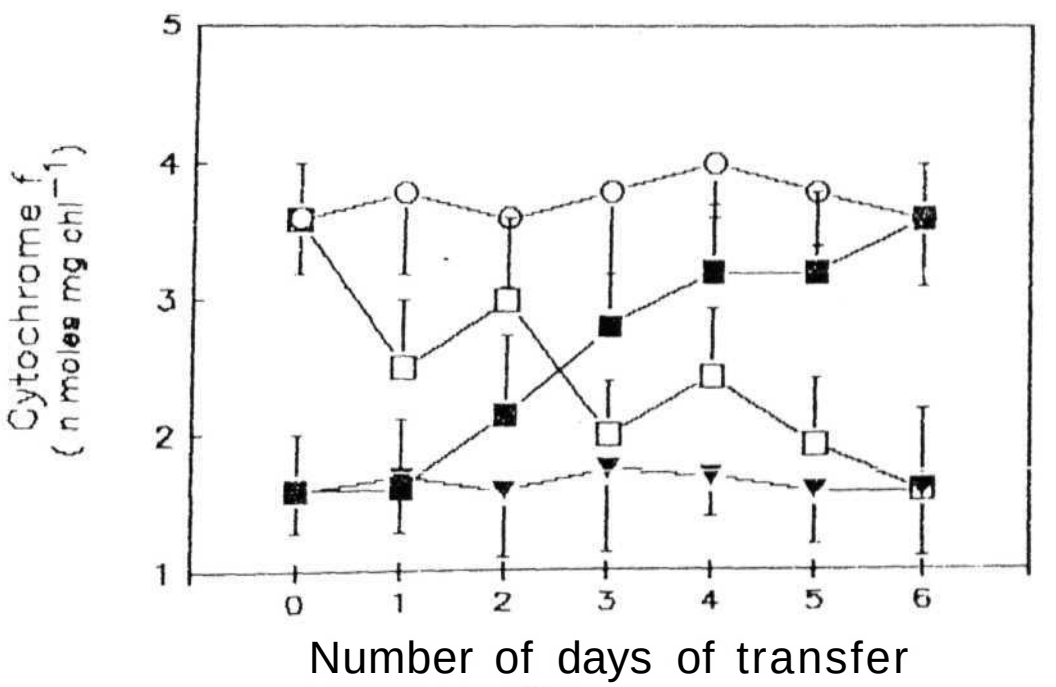
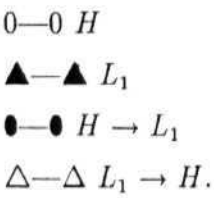
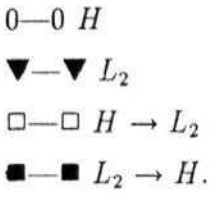

Cytochrome f
(n moles mg chl^{-1})
5
4
3
2
1
0
1
2
3
4
5
6
Number of days of transfer

Figure 3.16a. Light intensity effect on cytochrome / content in bundle sheath thylakoid membranes of *Amaranthus hypochondriacus* L.



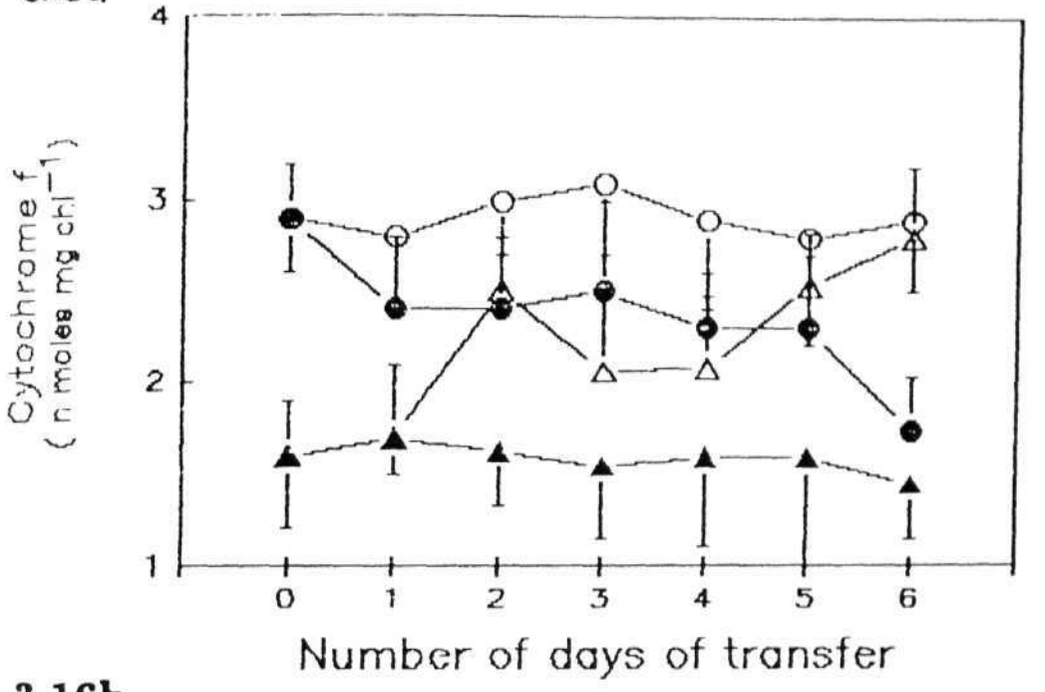
The results are average of three independent experiments.

Figure 3.16b. Effect of light intensity on cytochrome / content in bundle sheath thylakoid membranes of *Amaranthus hypochondriacus* L.



The results are average of three independent experiments.

3.16a


Cytochrome f
(n moles mg chl^{-1})
4
3
2
1
0 1 2 3 4 5 6
Number of days of transfer

3.16b

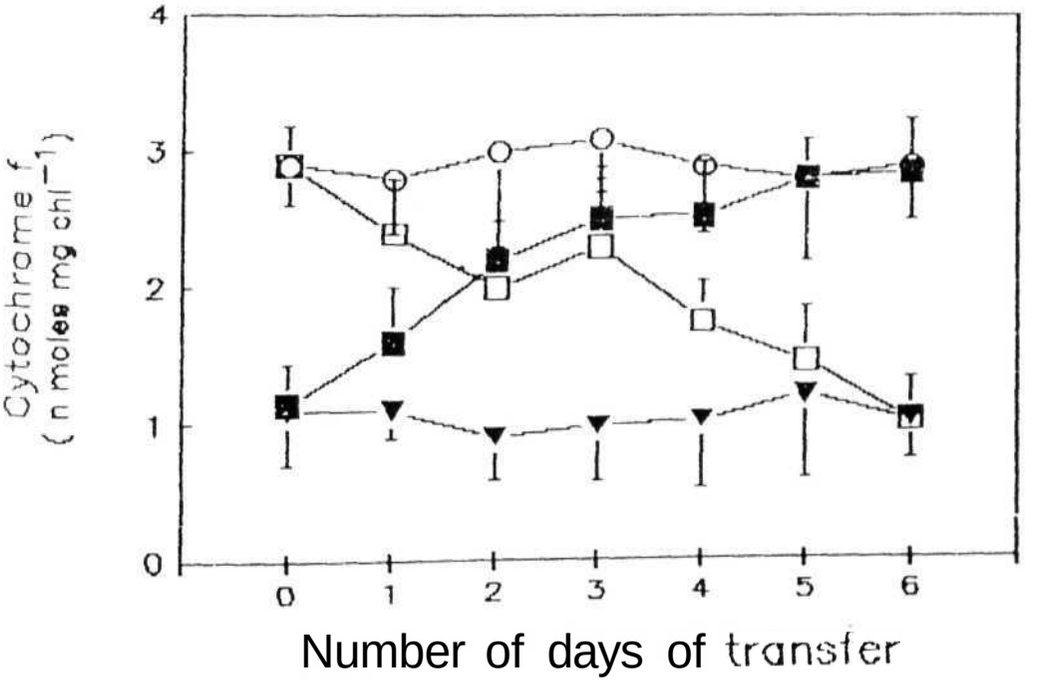

Cytochrome f
(n moles mg chl^{-1})
4
3
2
1
0
0 1 2 3 4 5 6
Number of days of transfer

Figure 3.17a. Effect of light intensity on chlorophyll/cytochrome f ratio in mesophyll thylakoid membranes of *Amaranthus hypochondriacus* L.

0—0 H

▲—▲ L_1

⬮—⬮ $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 3.17b. Modulation of chlorophyll/cytochrome f in mesophyll thylakoid membranes of *Amaranthus hypochondriacus.* L. under various light regimes.

0—0 H

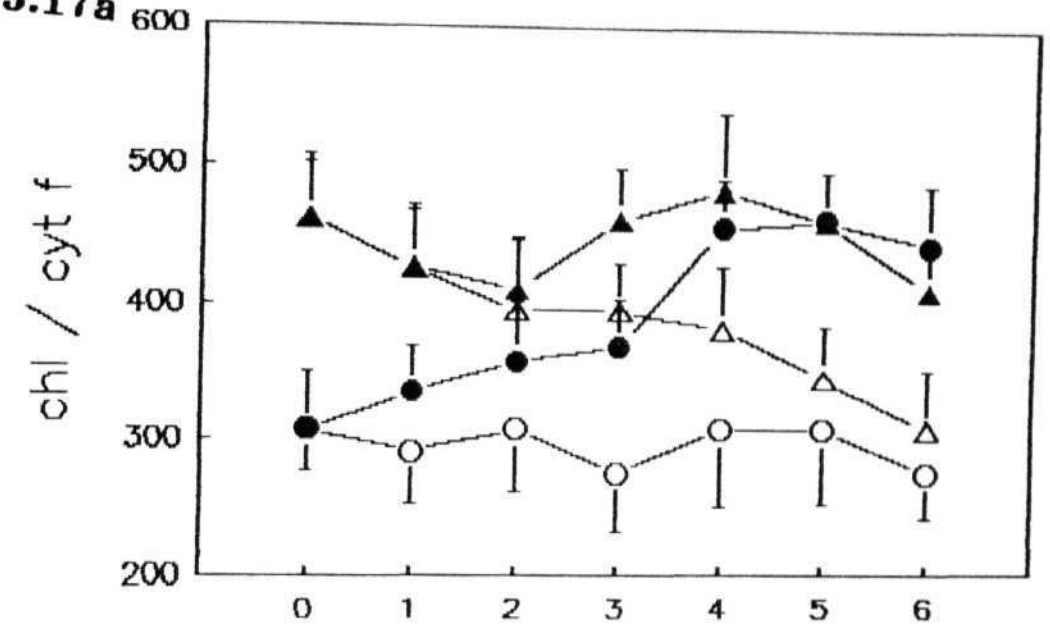
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

3.17a


600
500
400
300
200
chl / cyt f
0
1
2
3
4
5
6
Number of days of transfer

3.17b

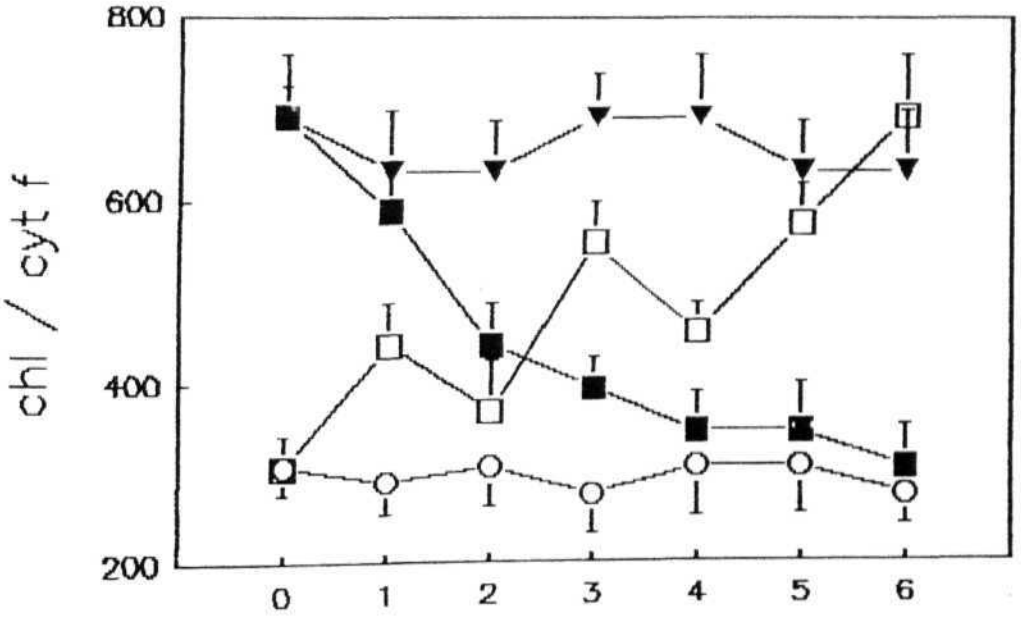
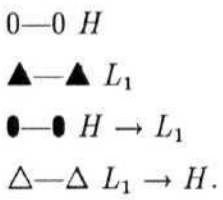
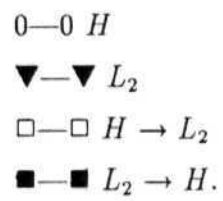

800
600
400
200
chl / cyt f
0
1
2
3
4
5
6
Number of days of transfer

Figure 3.18a. Effect of light intensity on chlorophyll/cytochrome f ratio in bundle sheath thylakoid membranes of *Amaranthus hypochondriacus* L.



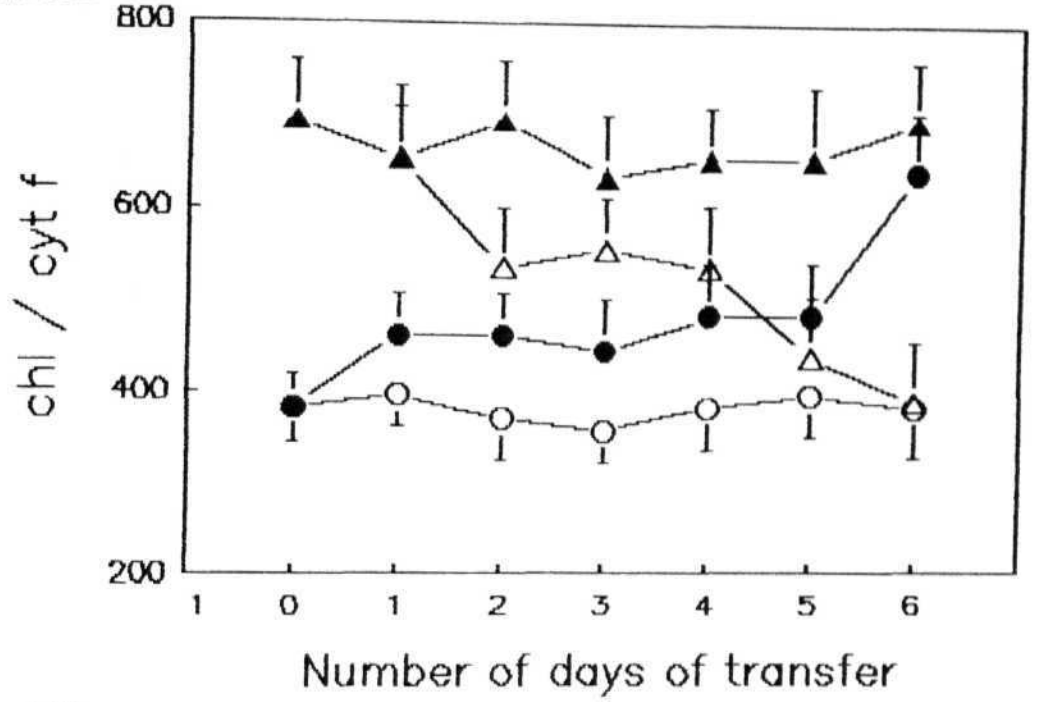
The results are average of three independent experiments.

Figure 3.18b. Modulation of chlorophyll/cytochrome f ratio in bundle sheath thylakoid membranes of *Amaranthus hypochondriacus* L. under various light regimes.

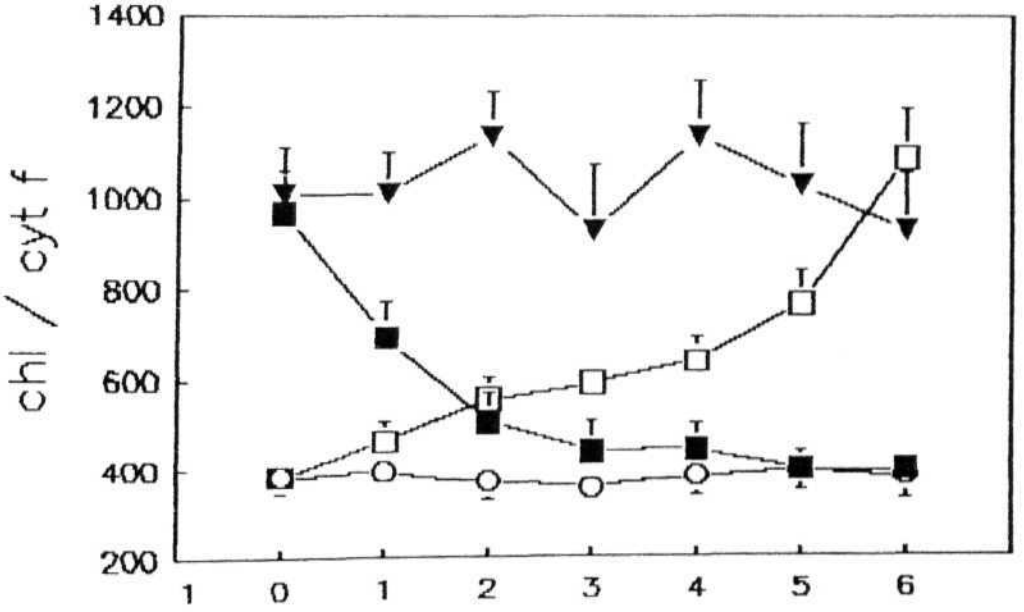


The results are average of three independent experiments.

3.18a


800
600
400
200
chl / cyt f
1 0 1 2 3 4 5 6
Number of days of transfer

3.18b


1400
1200
1000
800
600
400
200
chl / cyt f
1 0 1 2 3 4 5 6
Number of days of transfer

and 3.18b; Table 3.5). The ratio elevated in mesophyll (64% and 120%) and bundle sheath thylakoids (127% and 209%) of $H \rightarrow L_1$ and $H \rightarrow L_2$ plants (Figures 3.17a, 3.17b, 3.18a and 3.18b; Table 3.5). As observed earlier, the time taken for adjustment of the plants to altered light regimes was six days, without an initial lag.

Cytochrome f/P_{700} ratio remained constant in $H \rightarrow L_1$ and $H \rightarrow L_2$ *Amaranthus* plants in the mesophyll but declined in bundle sheath (Table 3.4; Figures 3.19a and 3.19b). The rise in the ratio was observed when low light grown plants were transferred to high irradiance in bundle sheath , in a time span of six days but remained unaltered in mesophyll thylakoid membranes (Figures 3.19a and 3.19b; Table 3.4).

The cytochrome / content decreased by 41% and 78% compared to // plants in $L\backslash$ and L_2 plants of *Eleusine* while 31% and 59% reduction was observed in similar plants of *Gomphrena* respectively (Figures 3.20a, 3.20b, 3.21a and 3.21b). In *Eleusine* cytochrome / content decreased by 41% and 78% of the content in *II* plants of $H \rightarrow L\backslash$ and $II \rightarrow L_2$ plants (Figures 3.20a and 3.20b), whereas the content increased by 69% and 361% in $L_1 \rightarrow H$ and $L_2 \rightarrow H$ plants (Table 3.4). The cytochrome / content modulation required four and eight days respectively , in response to one third and one tenth of normal light (Figure 3.20a and 3.20b). Cytochrome / content in thylakoid membranes of $H \rightarrow L_1$ and $H \rightarrow L_2$ *Gomphrena* plants decreased by 31% and 59%. The $L\backslash \rightarrow H$ and $L_2 \rightarrow$ // plants of *Gomphrena* exhibited 46% and 148% increased Cytochrome / content compared to the $L\backslash$ and L_2 plants in a time period of eight and ten days respectively (Figures 3.21a and 3.21b; Table 3.4).

Chlorophyll/cytochrome f ratio increased in $L\backslash$ and L_2 plants of *Eleusine* by 68% and 346%. In the plants grown at similar conditions, the ratio increased by 48% and 103%

Table 3.5: Effect of reduced irradiance on chlorophyll partitioning in the photosystems.

Species	Ratio				% decrease of control on acclimation		% increase of control on acclimation	
		H	L_1	L_2	$L_1 \rightarrow H$	$L_2 \rightarrow H$	$H \rightarrow L_1$	$H \rightarrow L_2$
Amaranthus								
Mesophyll	$\frac{Chl^{*}}{P_{700}^{**}}$	623±76	1015±158[a]	1413±190[a]	39	56	62	127
	$\frac{Chl^{*}}{Cyt\ f^{**}}$	338±48	555±76[a]	744±48[a]	39	55	64	120
Bundle sheath	$\frac{Chl^{*}}{P_{700}^{**}}$	689±80	797±196[d]	923±128[c]	14	25	16	34
	$\frac{Chl^{*}}{Cyt\ f^{**}}$	380±80	865±66[a]	1175±168[a]	56	68	127	209
Eleusine	$\frac{Chl^{*}}{Cyt\ f^{**}}$	311±33	523±18[a]	1234±380[a]	40	77	68	346
Gomphrena	$\frac{Chl^{*}}{Cyt\ f^{**}}$	326±34	485±58[a]	821±106[a]	32	60	48	103

*Chlorophyll; **Cytochrome f

The results are average of three independent experiments.

H : Thylakoid membranes from high light (2000 $\mu E\ m^{-2}s^{-1}$) grown plants.

L_1: Thylakoid membranes from low light (650 $\mu E\ m^{-2}s^{-1}$) grown plants.

L_2: Thylakoid membranes from low light (200 $\mu E\ m^{-2}s^{-1}$) grown plants.

$L_1 \rightarrow H$: Thylakoid membranes from low light (650 $\mu E\ m^{-2}s^{-1}$) grown plants after acclimation to high irradiance (2000 $\mu E\ m^{-2}s^{-1}$).

$L_2 \rightarrow H$: Thylakoid membranes from low light (200 $\mu E\ m^{-2}s^{-1}$) grown plants acclimated to high irradiance (2000 $\mu E\ m^{-2}s^{-1}$).

$H \rightarrow L_1$: Thylakoid membranes from high irradiance (2000 $\mu E\ m^{-2}s^{-1}$) grown plants acclimated to reduced light (650 $\mu E\ m^{-2}s^{-1}$).

$H \rightarrow L_2$: Thylakoid membranes from high irradiance (2000 $\mu E\ m^{-2}s^{-1}$) grown plants acclimated to reduced light (200 $\mu E\ m^{-2}s^{-1}$).

a = $p < 0.001$ c = $p < 0.02$ d = $p < 0.05$.

Figure 3.19a. Light intensity effect on cytochrome f/P_{700} ratio in bundle sheath thylakoid membranes of *Amaranthus hypochondriacus* L.

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 3.19b. Light intensity effect on cytochrome f/P_{700} ratio in bundle sheath thylakoid membranes of *Amaranthus hypochondriacus.* L.

0—0 H

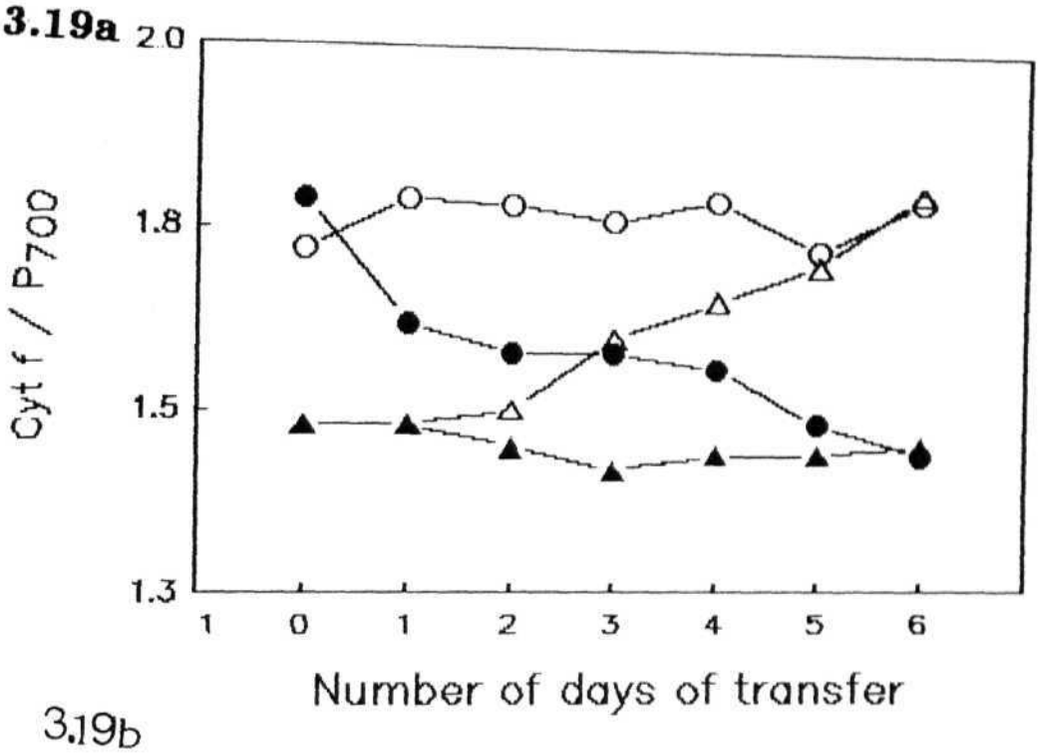
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

3.19a


2.0
1.8
1.5
1.3
Cyt f / P700
1 0 1 2 3 4 5 6
Number of days of transfer

3.19b

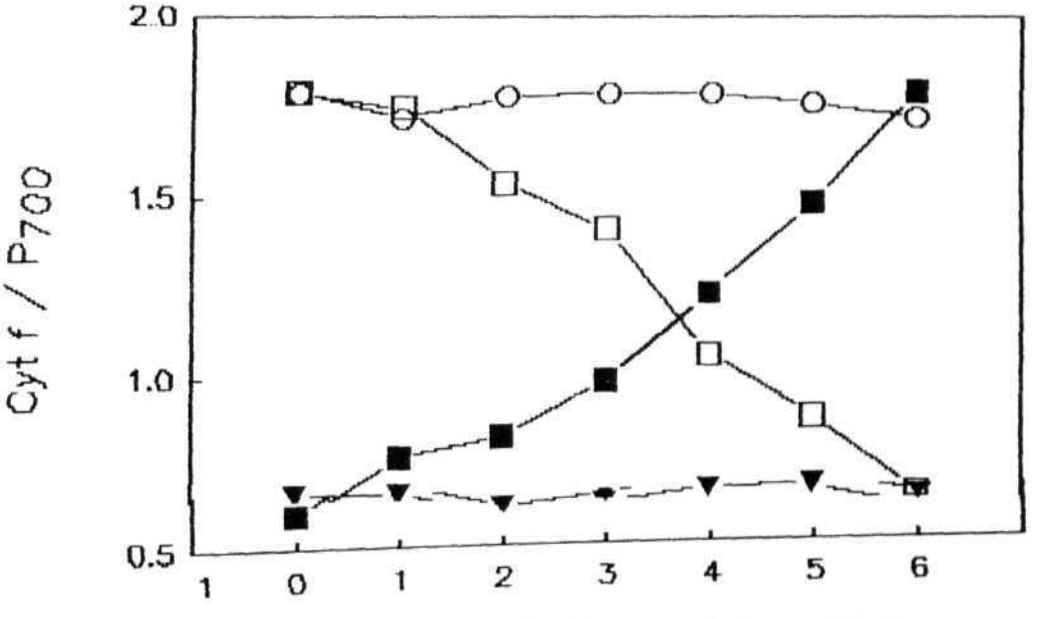

2.0
1.5
1.0
0.5
Cyt f / P700
1 0 1 2 3 4 5 6
Number of days of transfer

Figure 3.20a. Cytochrome f content in thylakoid membranes of *Eleusine coracana* under various light intensities.

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 3.20b. Cytochrome f content in thylakoid membranes of *Eleusine coracana* under various light intensities.

0—0 H

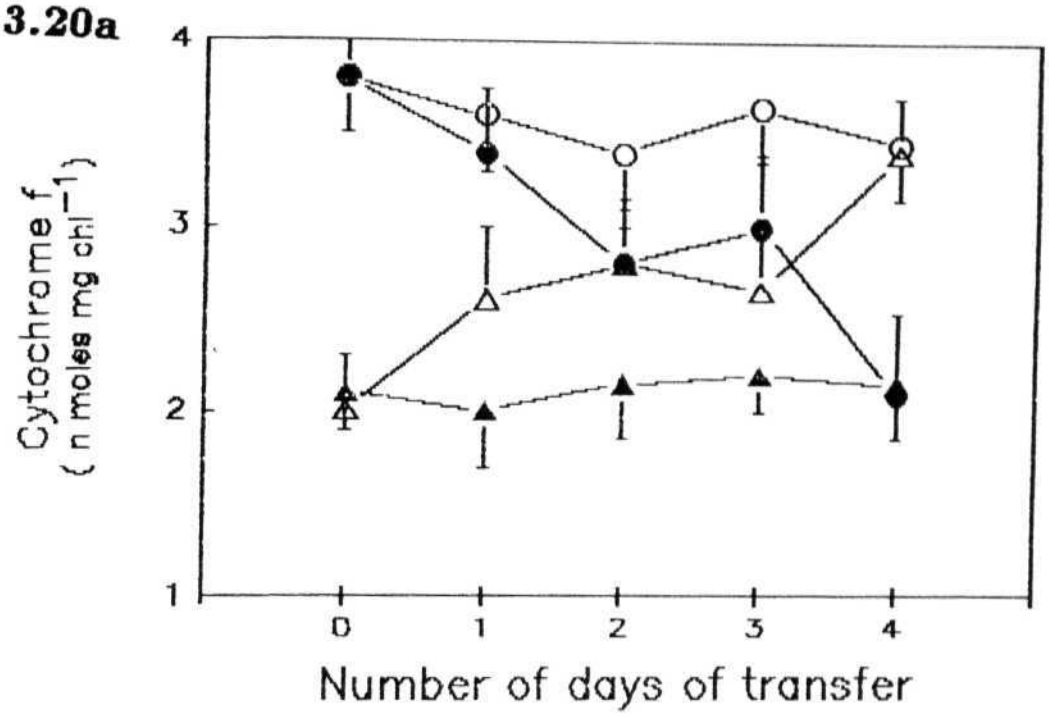
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three independent, experiments.

3.20a


Cytochrome f
(n moles mg chl^{-1})
4
3
2
1
0
1
2
3
4
Number of days of transfer

3.20b

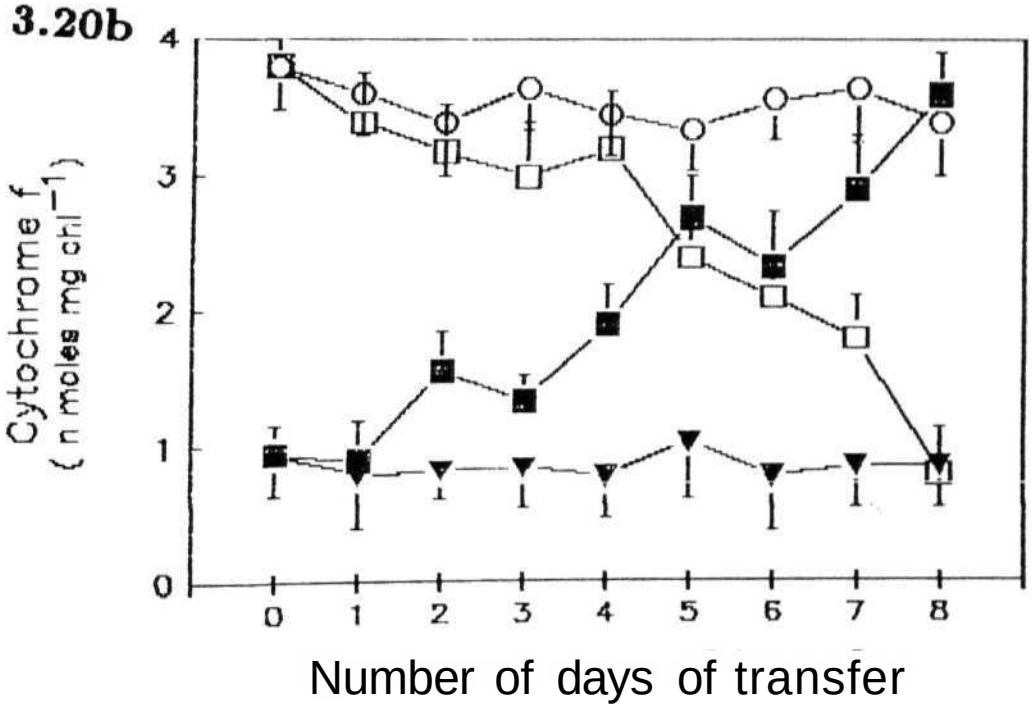

Cytochrome f
(n moles mg chl^{-1})
4
3
2
1
0
0
1
2
3
4
5
6
7
8
Number of days of transfer

Figure 3.21a. Light intensity effect on cytochrome f content in thylakoid membranes of *Gomphrena globosa.*

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 3.21b. Cytochrome f content in thylakoid membranes of *Gomphrena globosa* under various light intensities.

0—0 H

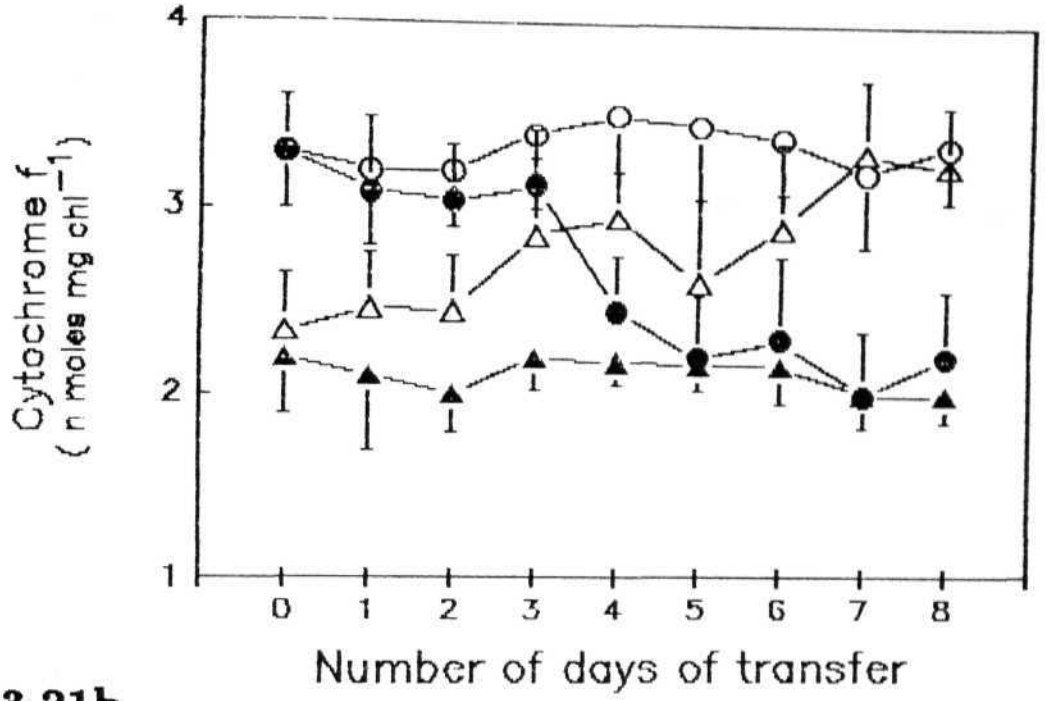
▼—▼ L_2

□—□ $H \rightarrow L_2$

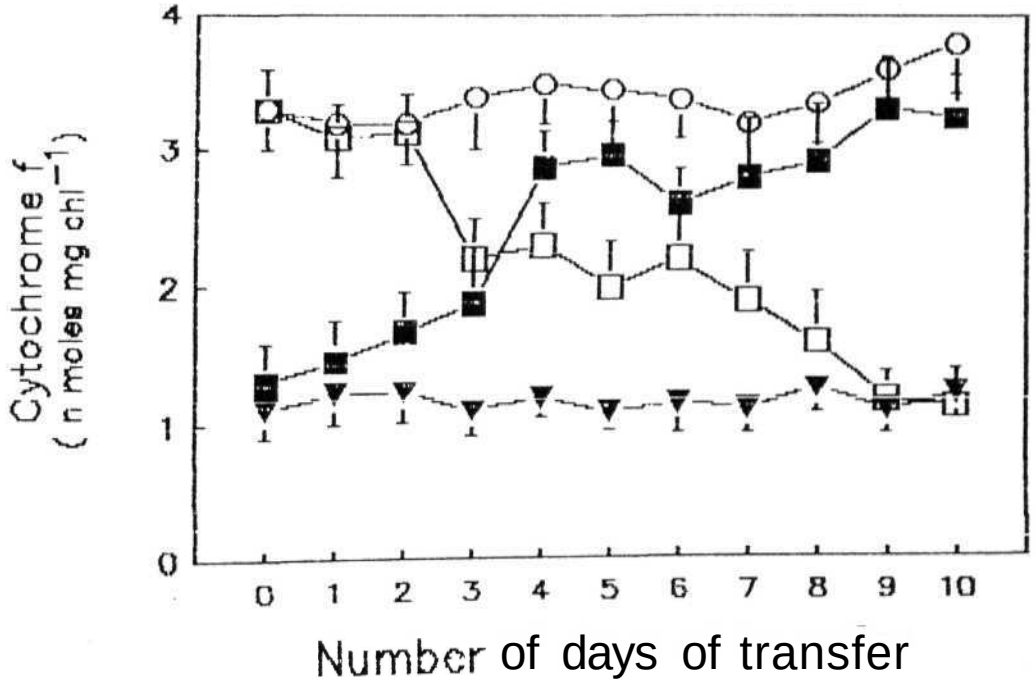
■—■ $L_2 \rightarrow H$.

The results arc average of three independent experiments.

3.21a


Cytochrome f
(n moles mg chl^{-1})
4
3
2
1
0 1 2 3 4 5 6 7 8
Number of days of transfer

3.21b


Cytochrome f
(n moles mg chl^{-1})
4
3
2
1
0
0 1 2 3 4 5 6 7 8 9 10
Number of days of transfer

in *Gomphrena* (Table 3.5). The number of chlorophyll molecules which served the PSI1 reaction centre increased in the plants grown under reduced irradiances in both *Eleusine* and *Gomphrena.* The increase was highest in *Eleusine* (Table 3.5; Figures **3.22a,** 3.22b 3.23a and 3.23b; Table 3.5). In $H \rightarrow L_1$ and $H \rightarrow L_2$ plants, Chlorophyll/cytochrome **f** ratio increased by 68% and 346% in *Eleusine* and 48% and 176% in *Gomphrena.* The ratio decreased by 40% and 78% in $L_1 \rightarrow H$ and $L_2 \rightarrow H$ plants of *Eleusine* while 32% and 69% increase was noticed in similar plants of *Gomphrena* respectively (Figures 3.22a, 3.22b, 3.23a and 3.23b). The percentage increase in the ratio did not correspond with percentage decrease in the ratio in both *Eleusine* and *Gomphrena* (Table 3.5). The time taken for such transfer studies correlated with electron transport rates and cytochrome / content in both the plants. As observed earlier for other functions and components of thylakoid membrane, cytochrome / content showed alterations after an initial lag of twenty four to forty eight hours in both *Eleusine* and *Gomphrena,* when plants were transferred from one light regime to another. The time lag in perceiving the light signal was absent in *Amaranthus.*

Oxygen evolution and Carbondioxide fixation rates:

Oxygen evolution rates decreased by 52% and 70% in L_1 and L_2 plants of *Amaranthus* (Figures 3.24a and 3.24b). $H \rightarrow L_1$ and $H \rightarrow L_2$ plants showed 52% and 70% reduction in the rates but $L_1 \rightarrow H$ and $L_2 \rightarrow H$ plants exhibited 111% and 220% increase respectively in a time period of six days, without any lag (Figures 3.24a and 3.24b). CO_2 fixation rates under various light regimes also showed similar trend. The CO_2 fixation rates in *Eleusine* and *Gomphrena* under various light regimes are presented in Tables 3.6 and 3.7 respectively (Tables 3.6 and 3.7). CO_2 fixation decreased by 51% and 71% in $H \rightarrow L_1$

Figure 3.22a. Light intensity effect on chlorophyll/cytochrome f ratio in thylakoid membranes of *Eleusine coracana.*

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 3.22b. Light intensity effect on chlorophyll/cytochrome f ratio in thylakoid membranes oi *Eleusine coracana.*

0—0 H

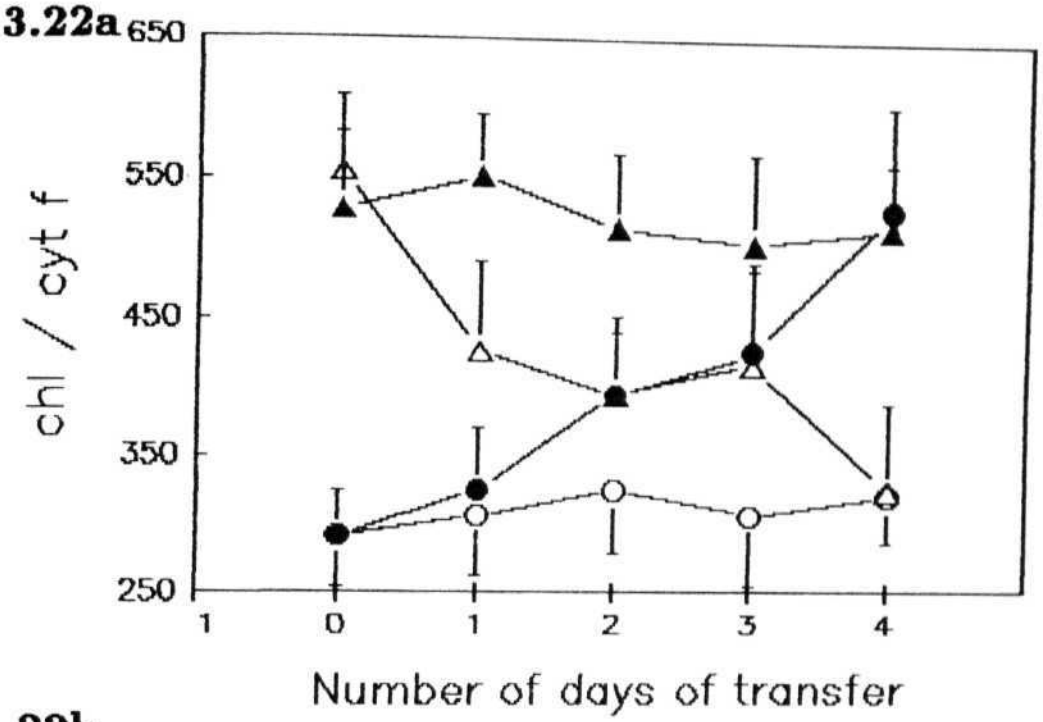
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

3.22a


650
550
450
350
250
chl / cyt f
1
0
1
2
3
4
Number of days of transfer

3.22b

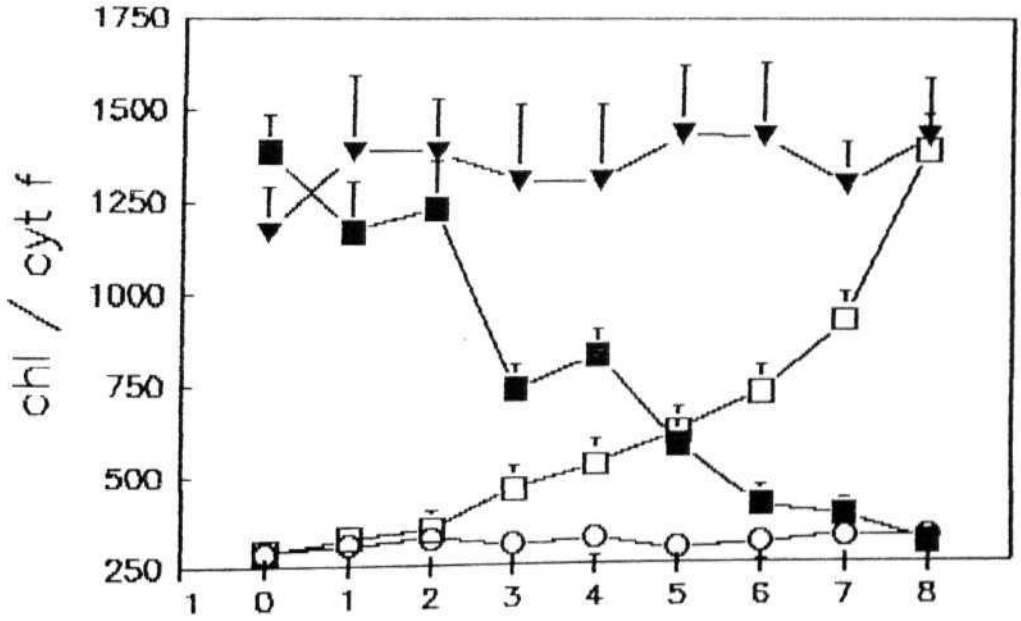

1750
1500
1250
1000
750
500
250
chl / cyt f
1
0
1
2
3
4
5
6
7
8
Number of days of transfer

Figure 3.23a. Light intensity effect on chlorophyll/cytochrome f ratio in thylakoid membranes of *Gomphrena globosa.*

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 3.23b. Light intensity effect on chlorophyll/cytochrome f ratio in thylakoid membranes of *Gomphrena globosa.*

0—0 H

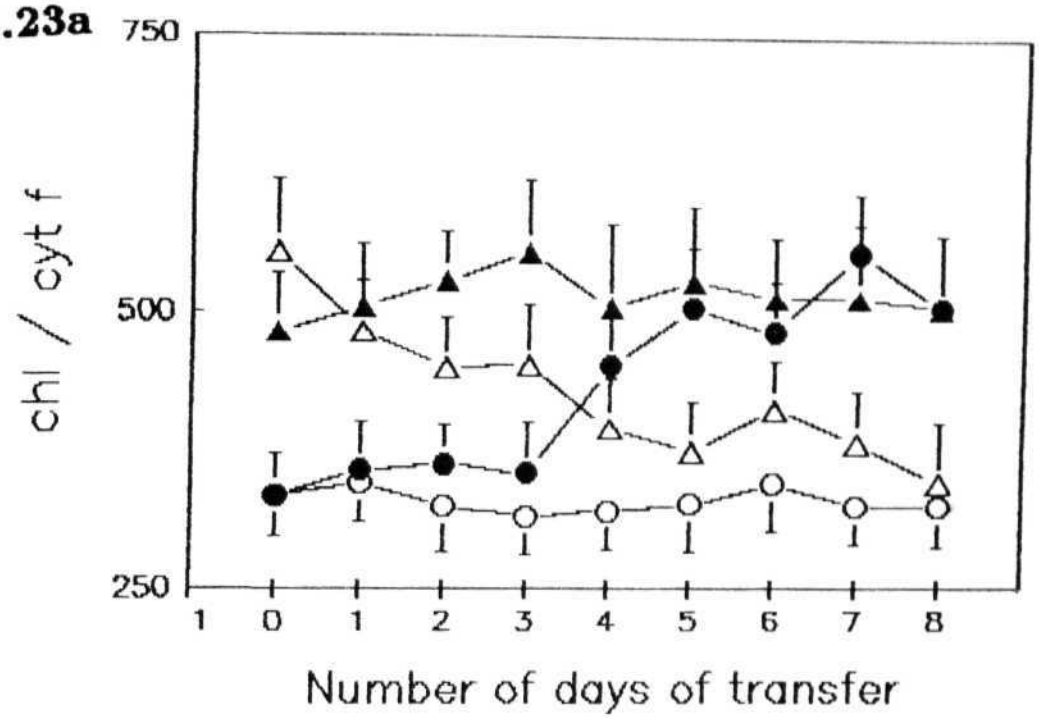
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

3.23a


750
500
250
chl / cyt f
1 0 1 2 3 4 5 6 7 8
Number of days of transfer

3.23b

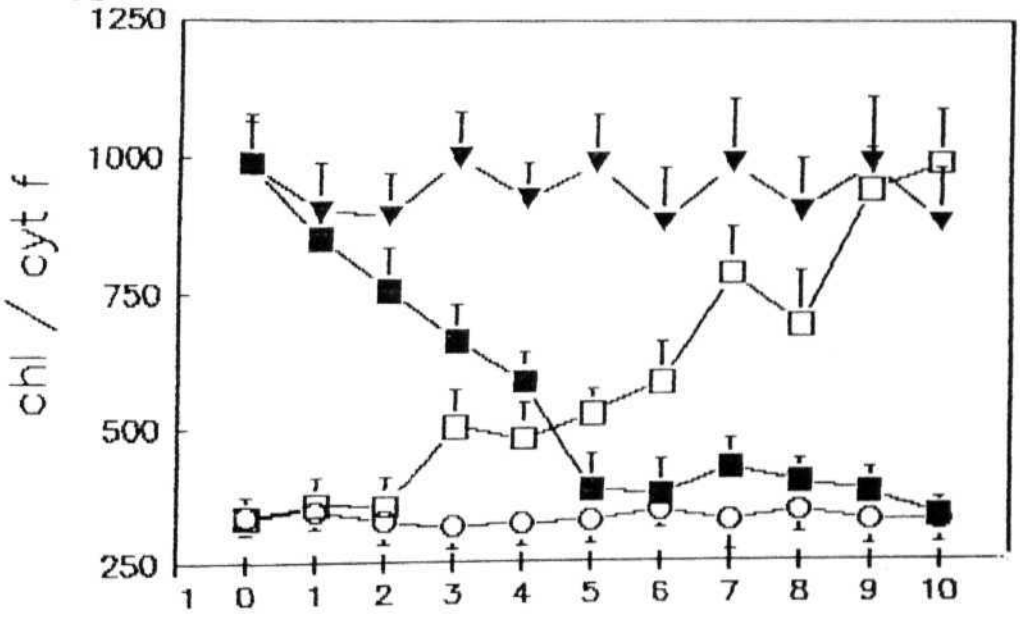
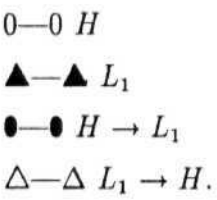
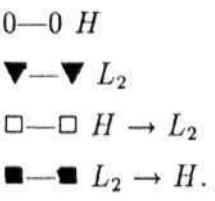

1250
1000
750
500
250
chl / cyt f
1 0 1 2 3 4 5 6 7 8 9 10
Number of days of transfer

Figure 3.24a. Light intensity effect on oxygen evolution rates in leaves of *Amaranthus hypochondriacus* L.



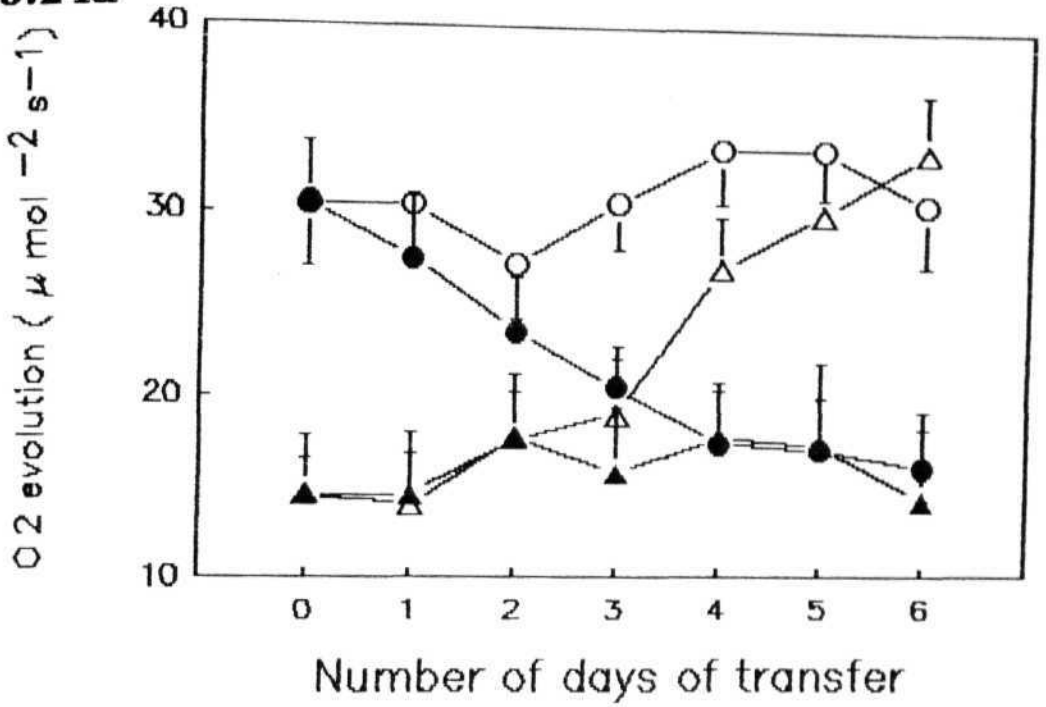
The results are average of three independent experiments.

Figure 3.24b. Light intensity effect on oxygen evolution rates in leaves of Amaranthus hypochondriacus L.



The results are average of three independent experiments.

3.24a


O 2 evolution (μ mol −2 s−1)
40
30
20
10
0
1
2
3
4
5
6
Number of days of transfer

3.24b

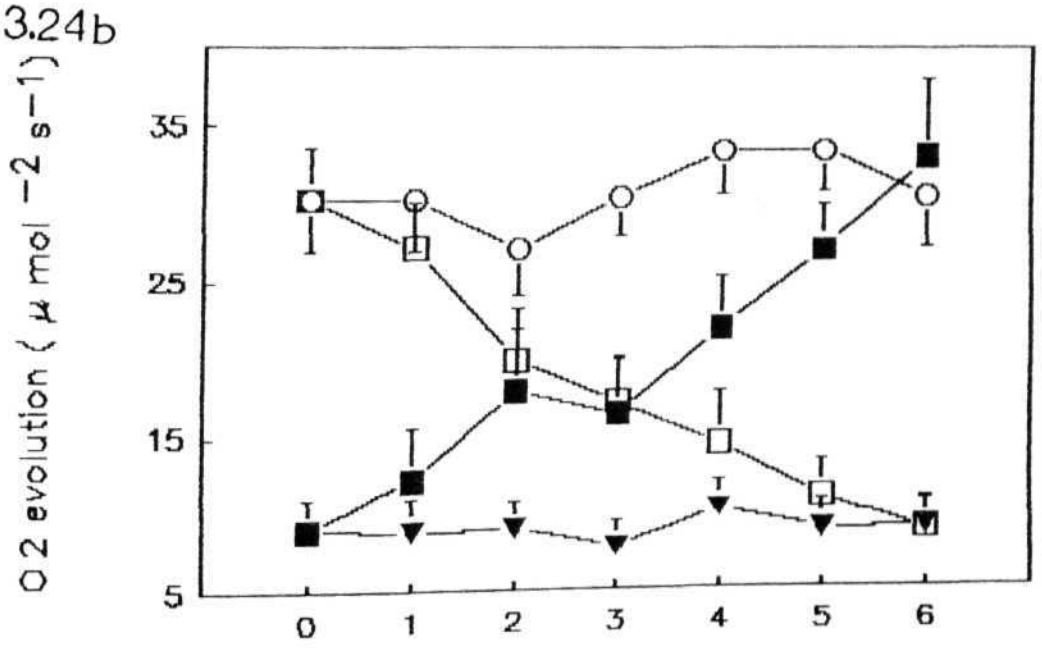

O 2 evolution (μ mol −2 s−1)
35
25
15
5
0
1
2
3
4
5
6
Number of days of transfer

Table 3.6: CO_2 Fixation rates during acclimation in *Eleusine coracana*

Growth condition	CO_2 Fixation rates (μmoles $m^{-2}s^{-1}$) Before acclimation	After acclimation	% Change of control
High light (2000 μE $m^{-2}s^{-1}$) (H)	42.4±5.6	44.6±5.8	
High light (650 μE $m^{-2}s^{-1}$) (L_1)	22.2±2.97	21.2±2.9	
High light (650 μE $m^{-2}s^{-1}$) (L_2)	16.6±0.12	16.6±0.9	
H $\rightarrow$ L_1	42.4±5.6	20.4±1.4[a]	51
H $\rightarrow$ L_2	42.4±5.6	12.2±0.2[a]	71
L_1 $\rightarrow$ H	21.2±2.9	44.3±4.5[a]	108
L_2 $\rightarrow$ H	16.6±0.13	45.0±6.3[a]	171

* Values are mean of 10 independent estimations.

a = $p < 0.001$

Table 3.7: CO_2 Fixation rates during acclimation in *Gomphrena globosa*

Growth condition	CO_2 Fixation rates (μmoles $m^{-2}s^{-1}$)		
	Before acclimation	After acclimation	% Change of control
High light (2000 μE $m^{-2}s^{-1}$) (H)	22.3±0.2	21.0±1.8	
Low light (650 μE $m^{-2}s^{-1}$) (L_1)	16.3±0.3	15.9±1.6	
Low light (200 μE $m^{-2}s^{-1}$) (L_2)	9.8±0.5	10.3±1.9	
$H \rightarrow L_1$	22.3±0.2	14.9±0.3[a]	33
$H \rightarrow L_2$	22.3±0.2	12.9±0.4[b]	42
$L_1 \rightarrow H$	15.4±0.3	22.2±3.0[a]	35
$L_2 \rightarrow H$	10.3±0.7	19.3±0.7[a]	86

Values are mean of 10 independent estimations.

a = $p < 0.001$ b = $p < 0.005$

and $H \rightarrow L_2$ plants of *Eleusine* and 33% and 42% decrease was observed in similar plants of *Gomphrena* respectively. The $L_1 \rightarrow H$ and $L_2 \rightarrow H$ plants exhibited 108% and 171% increased CO_2 fixation rates in *Eleusine* while 35% and 86% increased rates were observed in *Gomphrena* respectively. At a given reduced growth irradiance *Gomphrena* exhibited least reduction in CO_2 fixation followed by *Amaranthus* and *Eleusine* (Tables 3.6 and 3.7; Figure 3.24a and 3.24b).

C_4 Metabolism

C_4 plants are classified into three different subgroups depending on the mode of decarboxylating enzyme in the C_4 pathway. *Amaranthus* and *Eleusine* belong to NAD-ME C_4 type. *Gomphrena* belongs to NADP-ME type. The first enzyme which fixes carbondioxide in a C_4 plant is phosphoenol pyruvate Carboxylase. The CO_2 reacts with phosphoenol pyruvate to form OAA.

Phosphoenol pyruvate Carboxylase activity decreased by 49% and 81% of the control values in L_1 and L_2 plants of *Amaranthus.* The enzyme activity decreased by 73% and 81% in similar plants of *Eleusine* while 61% and 80% decrease was observed in *Gomphrena* L_1 and L_2 plants respectively (Tables 4.1, 4.2 and 4.3).

When // $\rightarrow L_1$ and $H \rightarrow L_2$ plants of *Amaranthus* were transferred from high irradiance to reduced irradiances phosphoenol pyruvate Carboxylase activity decreased by 49% and 81% of the control values, in a time period of three and five days respectively (Figures 4.1a and 4.1b). The enzyme levels increased by 197% and 434% of the L_1 and L_2 values respectively in $L\backslash \rightarrow H$ and $L_2 \rightarrow H$ plants to exhibit the activity observed in high light grown plants, in a time span of three and five days respectively (Figures 4.1a and 4.1b).

Table **4.1**: Effect of light intensity on key enzymes of C_4 metabolism in *Amaranthus hypochondriacus* L.

Enzymes	Enzyme activity from plants grown at different light intensities (μmoles mg $chl^{-1}h^{-1}$)			% decrease of control on acclimation		% increase of control on acclimation	
	H	L_1	L_2	H→L_1	H→L_2	L_1→H	L_2→H
PEP C[•]	2340±220	1176±138[a]	438±58[b]	49	81	197	434
As AT[••]	782±92	532±68[c]	182±26[b]	31	76	45	325
NAD-MDH[‡]	2111±280	1907±176[c]	1460±210[d]	10	30	11	45
NAD-ME[‡‡]	935±129	220±36[b]	102±18[a]	76	89	326	810
Al AT[#]	976±81	384±38[a]	266±31[a]	70	78	230	458
PPDK[##]	948±148	532±96[b]	298±36[a]	44	69	78	224

[•]Phosphoenol pyruvate carboxylase.

[••]Aspartate amino transferase.

[‡]NAD-Malate dehydrogenase.

[‡‡]NAD-Malic enzyme.

[#]Alanine amino transferase.

[##]Pyruvate orthophosphate dikinase.

The results are average of 10 independent estimations

H : plants grown at 2000 μE $m^{-2}s^{-1}$.

L_1 : plants grown at 650μE $m^{-2}s^{-1}$.

L_2 : plants grown at 200 μE $m^{-2}s^{-1}$.

H→L_1: plants grown at high irradiance (2000 μE $m^{-2}s^{-1}$) acclimated to reduced light (650 μE $m^{-2}s^{-1}$).

H→L_2: plants grown at high irradiance (2000 μE $m^{-2}s^{-1}$) acclimated to reduced light (200 μE $m^{-2}s^{-1}$).

L_1→H: plants grown at reduced irradiance (650 μE $m^{-2}s^{-1}$) acclimated to high irradiance (2000 μE $m^{-2}s^{-1}$).

L_2→H: plants grown at reduced irradiance (200 μE $m^{-2}s^{-1}$) acclimated to high irradiance (2000 μE $m^{-2}s^{-1}$).

a = $p < 0.001$ b = $p < 0.005$ c = $p < 0.02$ d = $p < 0.05$.

Table 4.2: Effect of light intensity of key enzymes of C_4 metabolism in *Eleusine coracana*

Enzymes	Enzyme activity from plants grown at different light intensities (μmoles mg chl^{-1} h^{-1})			% decrease of control on acclimation		% increase of control on acclimation	
	H	L_1	L_2	H→L_1	H→L_2	L_1→H	L_2→H
PEP C•	1509±250	399±70^b	285±40^b	73	81	278	429
As AT••	1104± 98	421±53^a	274±42^a	61	78	162	363
NAD-MDH‡	2793±534	2210±234^b	1825±290^c	20	33	26	53
NAD-ME‡‡	968±160	352±68^a	175± 68^a	64	82	175	453
Al AT#	836± 90	341±46^b	236± 48^a	59	72	145	262
PPDK##	1376±251	478±59^b	312± 28^b	65	77	187	341

•Phosphoenol pyruvate carboxylase.

••Aspartate amino transferase.

‡NAD-Malate dehydrogenase.

‡‡NAD-Malic enzyme.

#Alanine amino transferase.

##Pyruvate orthophosphate dikinase.

The results are average of 10 independent estimations.

H : plants grown at 2000 μE $m^{-2}s^{-1}$.

L_1 : plants grown at 650 μE $m^{-2}s^{-1}$.

L_2 : plants grown at 200 μE $m^{-2}s^{-1}$.

H→L_1: plants grown at high irradiance (2000 μE $m^{-2}s^{-1}$) acclimated to reduced light (650 μE $m^{-2}s^{-1}$).

H→L_2: plants grown at high irradiance (2000 μE $m^{-2}s^{-1}$) acclimated to reduced light (200 μE $m^{-2}s^{-1}$).

L_1→H: plants grown at reduced irradiance (650 μE $m^{-2}s^{-1}$) acclimated to high irradiance (2000 μE $m^{-2}s^{-1}$).

L_2→H: plants grown at reduced irradiance (200 μE $m^{-2}s^{-1}$) acclimated to high irradiance (2000 μE $m^{-2}s^{-1}$).

a = $p < 0.001$ b = $p < 0.005$ c = $p < 0.02$

Table 4.3: Effect of light intensity of key enzymes of C_4 metabolism in *Gomphrena globosa*

Enzymes	Enzyme activity from plants grown at different light intensities (μmoles mg chl^{-1}h^{-1})			% decrease of control on acclimation		% increase of control on acclimation	
	H	L_1	L_2	H→L_1	H→L_2	L_1→H	L_2→H
PEP C•	3096±650	1180±172[b]	607±96[b]	61	80	162	410
NADP-MDH■	880± 98	588±46[b]	341±56[b]	33	61	49	157
NADP-ME■■	948±145	247±39[a]	112±56[a]	73	88	283	746
PPDK##	1070±150	478±49[b]	225±26[b]	50	78	100	375

•Phosphoenol pyruvate carboxylase. ■NADP-Malate dehydrogenase.

■■NADP-Malic enzyme. ##Pyruvate orthophosphate dikinase.

The results are average of 10 independent estimations.

H : plants grown at 2000 μE $m^{-2}s^{-1}$.

L_1 : plants grown at 650 μE $m^{-2}s^{-1}$.

L_2 : plants grown at 200 μE $m^{-2}s^{-1}$.

H→L_1: plants grown at high irradiance (2000 μE $m^{-2}s^{-1}$) acclimated to reduced light (650 μE $m^{-2}s^{-1}$).

H→L_2: plants grown at high irradiance (2000 μE $m^{-2}s^{-1}$) acclimated to reduced light (200 μE $m^{-2}s^{-1}$).

L_1→H: plants grown at reduced irradiance (650 μE $m^{-2}s^{-1}$) acclimated to high irradiance (2000 μE $m^{-2}s^{-1}$).

L_2→H: plants grown at reduced irradiance (200 μE $m^{-2}s^{-1}$) acclimated to high irradiance (2000 μE $m^{-2}s^{-1}$).

a = $p < 0.001$ b = $p < 0.005$

Figure 4.1a. Effect of light intensity on phosphoenol pyruvate Carboxylase activity in *Amaranthus hypochondriacus* L.

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 4.1b. Effect of light intensity on phosphoenol pyruvate Carboxylase activity in *Amaranthus hypochondriacus* L.

0—0 H

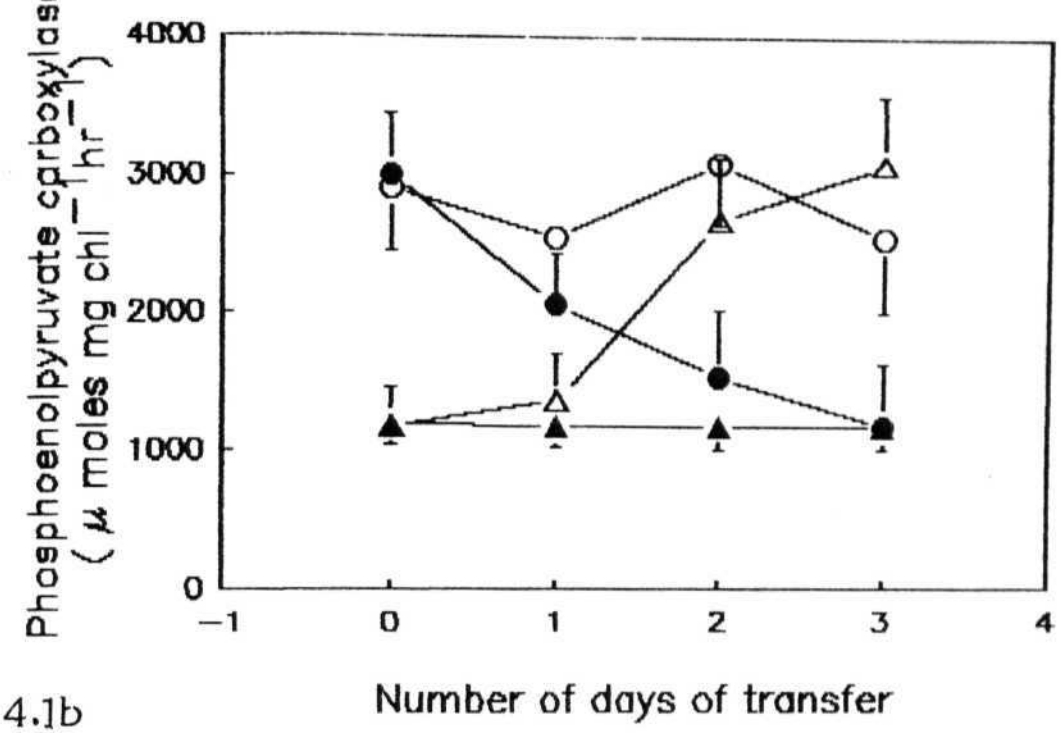
▼—▼ L_2

□—□ $H \rightarrow L_2$

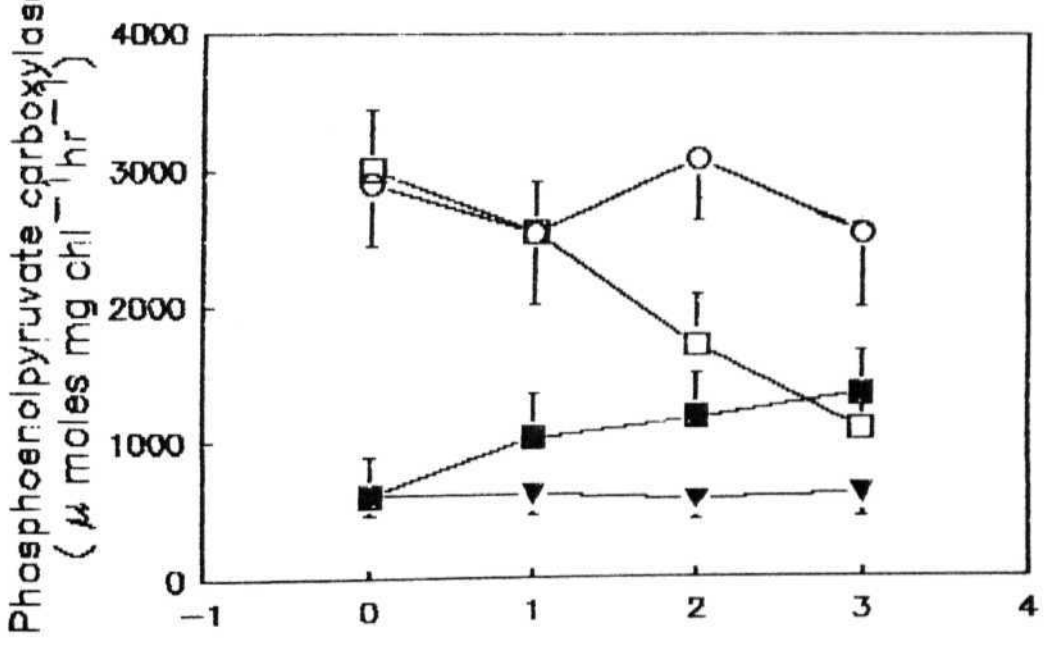
■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

4.1a


Phosphoenolpyruvate carboxylase
(μ moles mg chl⁻¹ hr⁻¹)
4000
3000
2000
1000
0
−1
0
1
2
3
4
Number of days of transfer

4.1b


Phosphoenolpyruvate carboxylase
(μ moles mg chl⁻¹ hr⁻¹)
4000
3000
2000
1000
0
−1
0
1
2
3
4
Number of days of transfer

The enzyme activity decreased by 73% and 81% in *Eleusine,* 61% and 80% in *Gomphrena* when H —* L_1 and $H \rightarrow L_2$ plants were transferred from high to lowered irradiances. The enzyme activity increased by 278% and 429% in *Eleusine,* 162% and 410% in *Gomphrena,* when plants grown under reduced irradiance were transferred to high irradiances (Figures 4.2a, 4.2b, 4.3a and 4.3b). The percentage decrease in enzyme activity did not correspond with the percentage increase in enzyme activity when $H \rightarrow L_1$ and $H \rightarrow L_2$ plants were transferred to reduced irradiances and $L_1 \rightarrow H$ and $L_2 \rightarrow H$ plants were transferred to high intensity in *Amaranthus, Eleusine* and *Gomphrena.*(Tables4.1, 4.2 and 4.3).-

OAA is converted into aspartate by reversible amino transferase in the cytosol of mesophyll cells in NAD-ME type C_4 plants *Amaranthus* and *Eleusine.* Aspartate aminotransferase activity decreased in L_1 and L_2 plants of *Amaranthus* (31% and 76%) and *Eleusine* (61% and 78%) compared to that of H plants (Figures 4.4a, 4.4b, 4.5a and 4.5b; Tables 4.1 and 4.2). When $H \rightarrow L_1$ and $H \rightarrow L_2$ plants were transferred to reduced irradiances the enzyme activity decreased by 31% and 76% in *Amaranthus,* whereas 61% and 78% decline was found in *Eleusine* respectively. The $L_1 \rightarrow$ // and $L_2 \rightarrow H$ plants on transfer to high irradiances showed 45% and 325% increase in the enzyme activity in *Amaranthus* while 162% and 363% increase was observed in *Eleusine.* The $H \rightarrow L_1$ and $L_1 \rightarrow H$ plants exhibited alterations in enzyme activity in three days while in $H \rightarrow L_2$, $L_2 \rightarrow H$ plants, changes in the enzyme activity was observed in five days (Figures 4.4a, 4.4b, 4.5a and 4.5b). The enzyme phosphoenol pyruvate Carboxylase was found to be more susceptible to the changes in irradiance compared to aspartate aminotransferase. (Tables 4.1 and 4.2).

Figure 4.2a. Effect of light intensity on phosphoenol pyruvate Carboxylase activity in *Eleusine coracana.*

0—0 H

▲—▲ L_1

●—● $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 4.2b. Effect of light intensity on phosphoenol pyruvate Carboxylase activity in *Eleusine coracana.*

0—0 H

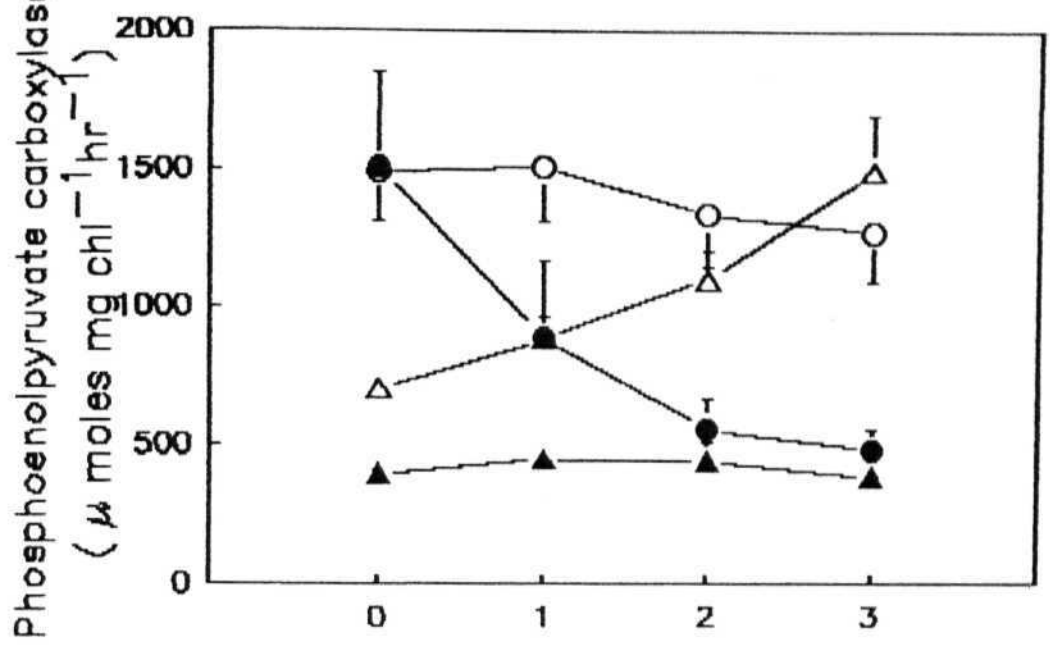
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

4.2a


Phosphoenolpyruvate carboxylase
(μ moles mg chl^{-1}hr^{-1})
2000
1500
1000
500
0
0
1
2
3
Number of days of transfer

4.2b

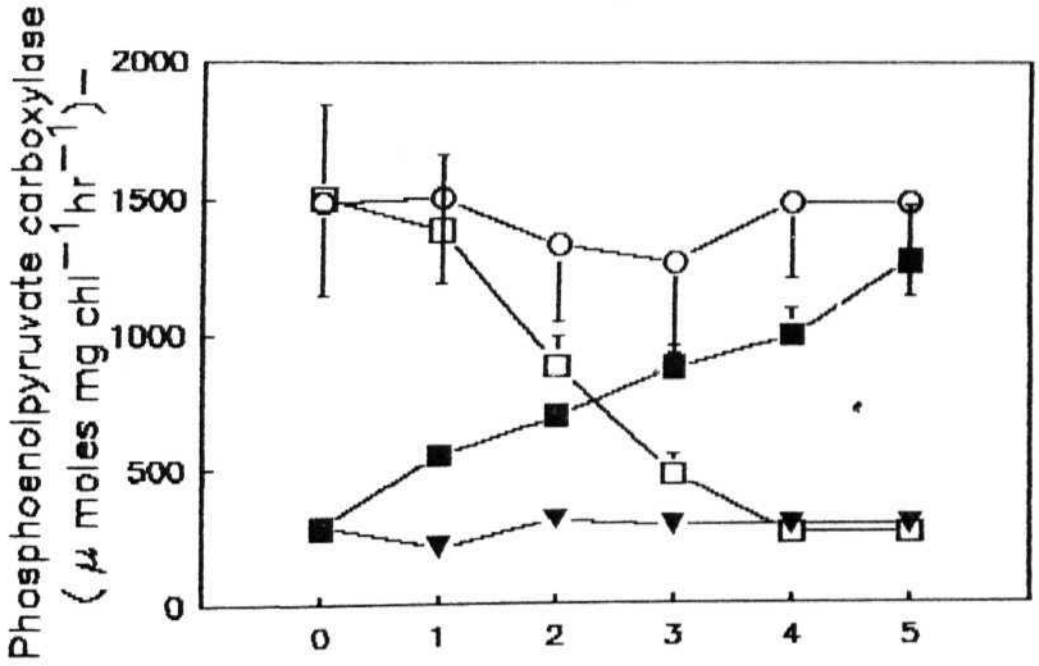

Phosphoenolpyruvate carboxylase
(μ moles mg chl^{-1}hr^{-1})
2000
1500
1000
500
0
0
1
2
3
4
5
Number of days of transfer

Figure 4.3a. Light intensity effect on phosphoenol pyruvate Carboxylase activity in *Gomphrena globosa.*

0—0 H

▲—▲ L_1

⬮—⬮ $H \rightarrow L_1$

△—△ $L_1 \rightarrow H$.

The results are average of three independent experiments.

Figure 4.3b. Light intensity effect on phosphoenol pyruvate Carboxylase activity in *Gomphrena globosa.*

0—0 H

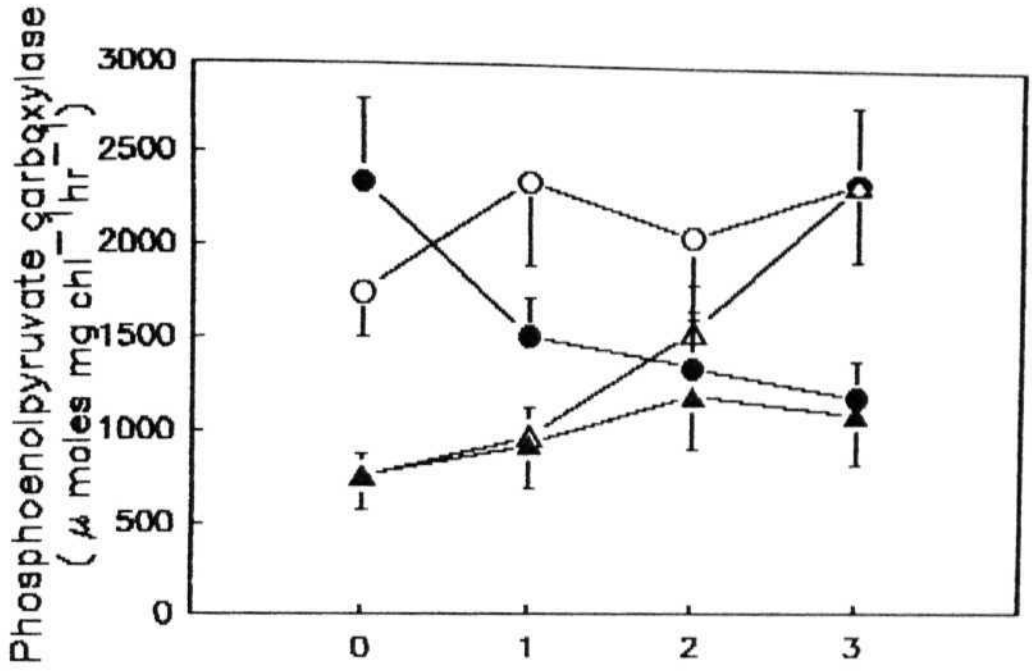
▼—▼ L_2

□—□ $H \rightarrow L_2$

■—■ $L_2 \rightarrow H$.

The results are average of three independent experiments.

4.3a


Phosphoenolpyruvate carboxylase
(μ moles mg chl^{-1} hr^{-1})
3000
2500
2000
1500
1000
500
0
0
1
2
3
Number of days of transfer

4.3b

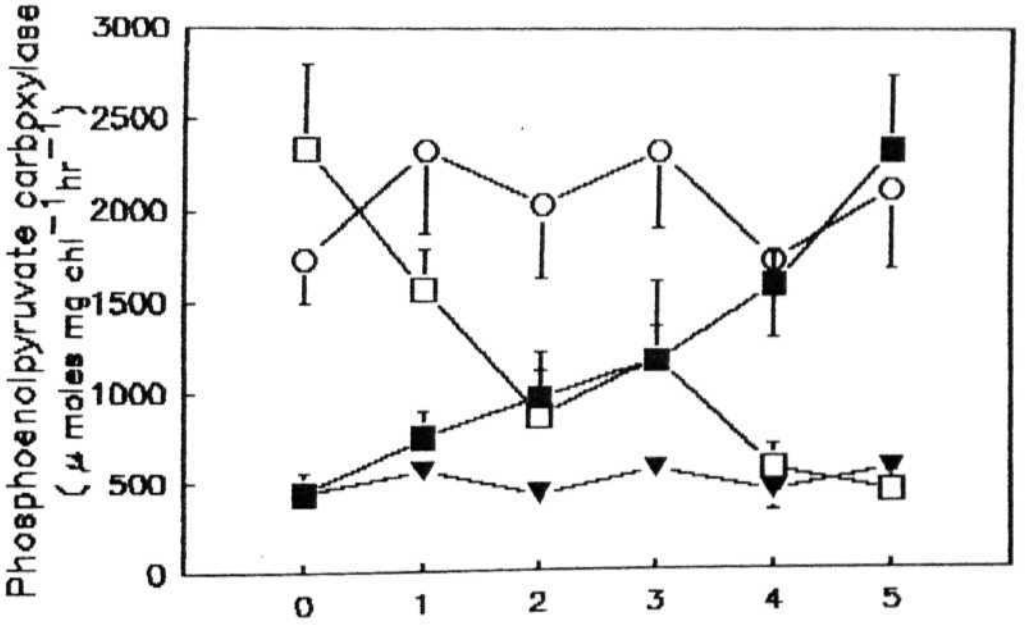
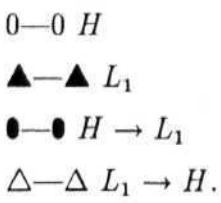
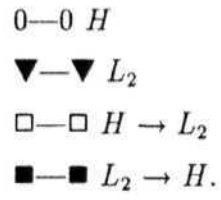

Phosphoenolpyruvate carboxylase
(μ moles mg chl^{-1} hr^{-1})
3000
2500
2000
1500
1000
500
0
0
1
2
3
4
5
Number of days of transfer

Figure 4.4a. Effect of light intensity on aspartate amino transferase activity in *Amaranthus hypochondriacus* L.



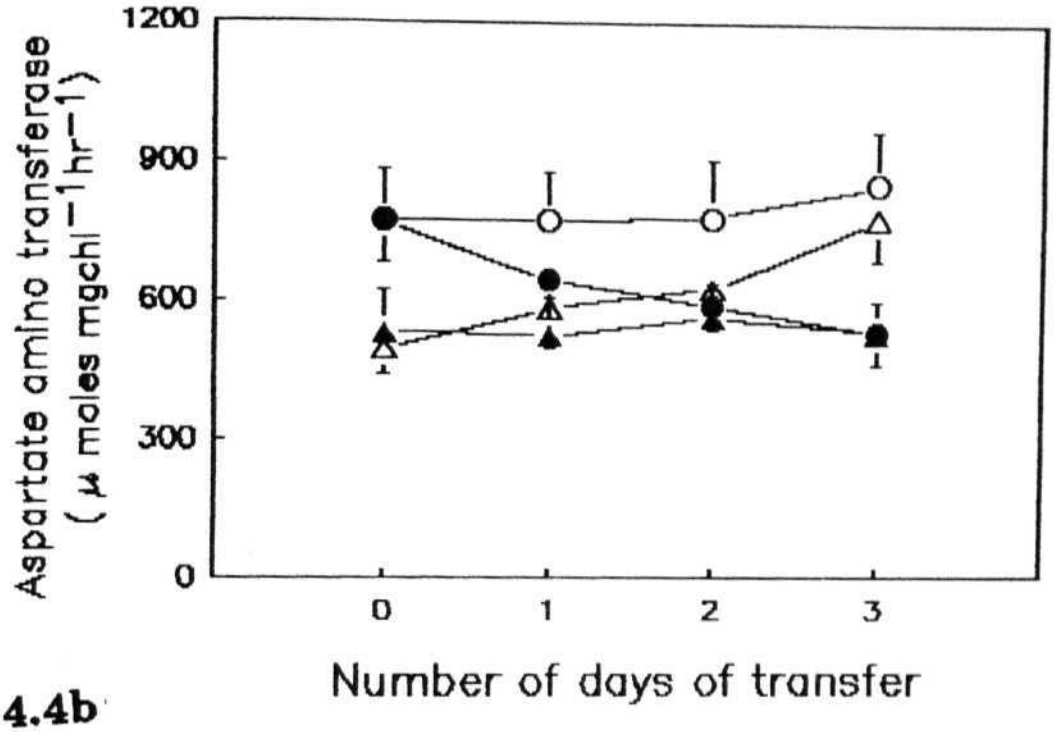
The results are average of three independent experiments.

Figure 4.4b. Effect of light intensity on aspartate amino transferase activity in *Amaranthus hypochondriacus* L.



The results are average of three independent experiments.

4.4a


Aspartate amino transferase
(μ moles mgchl⁻¹hr⁻¹)
1200
900
600
300
0
0
1
2
3
Number of days of transfer

4.4b

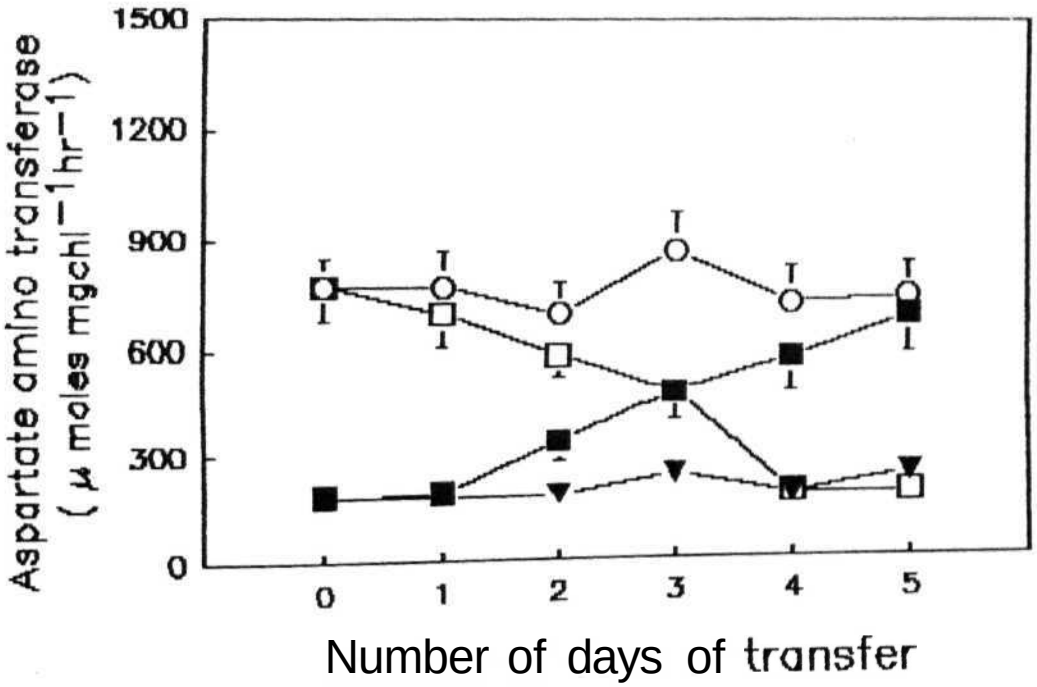
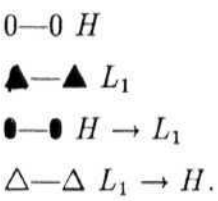
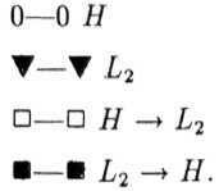

Aspartate amino transferase
(μ moles mgchl⁻¹hr⁻¹)
1500
1200
900
600
300
0
0
1
2
3
4
5
Number of days of transfer

Figure 4.5a. Effect of light intensity on aspartate amino transferase activity in *Eleusine coracana.*



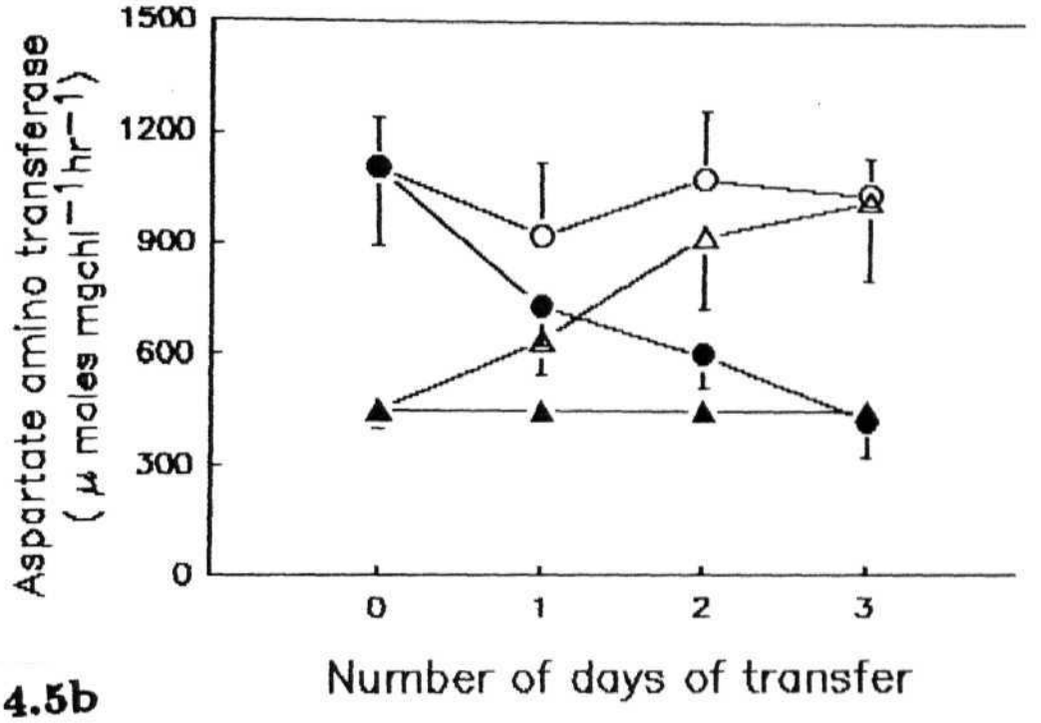
The results are average of three independent experiments.

Figure 4.5b. Effect of light intensity on aspartate amino transferase activity in *Eleusine coracana.*

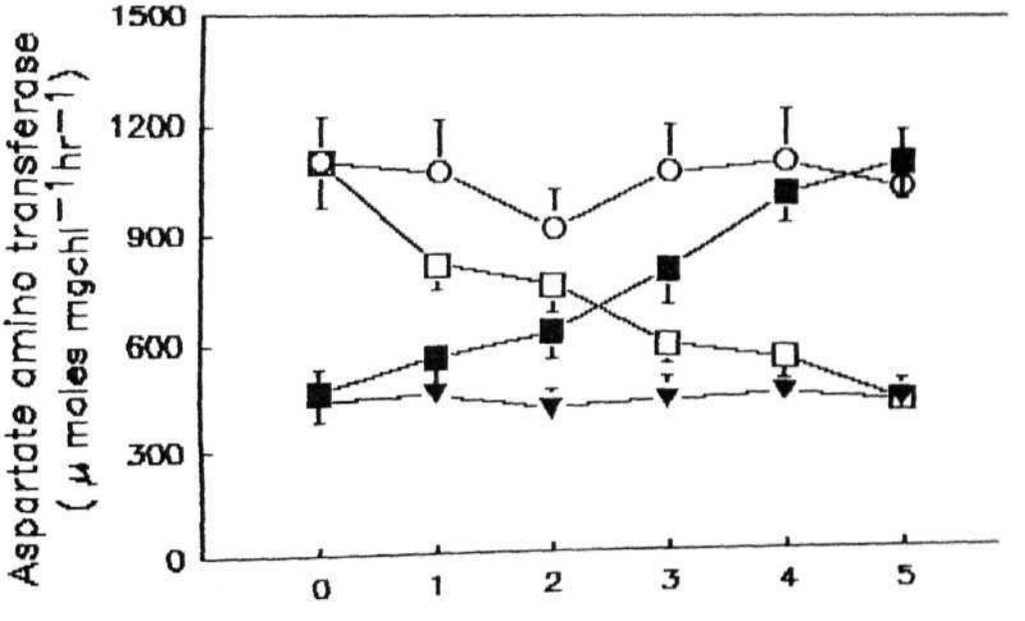


The results are average of three independent experiments.

4.5a


Aspartate amino transferase
(μ moles mgchl⁻¹hr⁻¹)
1500
1200
900
600
300
0
0
1
2
3
Number of days of transfer

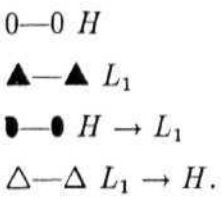
4.5b


Aspartate amino transferase
(μ moles mgchl⁻¹hr⁻¹)
1500
1200
900
600
300
0
0
1
2
3
4
5
Number of days of transfer

OAA is converted into malate by the enzyme NAD-Malate dehydrogenase in (NAD-MDH) *Amaranthus* and *Eleusine*. NAD-MDH activity decreased by 10% and 30% in L_1 and L_2 plants of *Amaranthus* compared to H plants. Under similar experimental conditions L_1 and L_2 plants of *Eleusine* showed 30% and 33% reduction in NAD-MDH activity. The NAD-ME activity declined (33% and 61%) in *Gomphrena* plants grown under lowered light levels. The enzyme activity decreased by 10% in *Amaranthus* and 20% in *Eleusine* when $H \rightarrow L_1$ plants were transferred to suboptimal light whereas the activity increased by 30%, and 26% in $L_1 \rightarrow H$ plants of *Amaranthus* and *Eleusine* respectively in a time period of three days (Figures 4.6a and 4.7a). When $H \rightarrow L_2$ plants were transferred from higher to lowered irradiances, 30% and 26% decrease in the enzyme activity was observed in *Amaranthus* and *Eleusine* respectively and the activity increased by 45% and 53 in the $L_2 \rightarrow H$ plants of *Amaranthus* and *Eleusine* on transfer to high irradiances, in a time period of five days (Figures 4.6b and 4.7b). NADP-MDH activity decreased in $H \rightarrow L_1$ and $H \rightarrow L_2$ plants by 33% and 63% whereas the activity increased in $L_1 \rightarrow H$ and $L_2 \rightarrow H$ plants by 49% and 157% (Figures 4.8a and 4.8b). NADP-MDH activity was reduced in *Gomphrena* plants to a greater extent compared to NAD-MDH activity in *Amaranthus* and *Eleusine* in response to limiting light (Tables 4.1, 4.2 and 4.3).

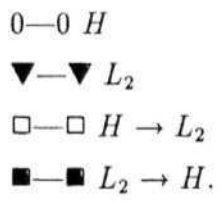
Malate is converted into pyruvate with the release of CO_2 (for fixation by Rubisco) by NAD-Malic enzyme in *Amaranthus* and *Eleusine* and NADP-Malic enzyme in *Gom~phrena*. NAD-ME activity decreased by 76% and 89% in L_1 and L_2 plants of *Amaranthus* while only 64% and 82% decline was observed in similar plants of *Eleusine* respectively. When $H \rightarrow L_1$ plants were transferred to lowered irradiance NAD-ME activity decreased

Figure 4.6a. Effect of varying light intensity on malate dehydrogenase activity in *Amaranthus hypochondriacus* L.



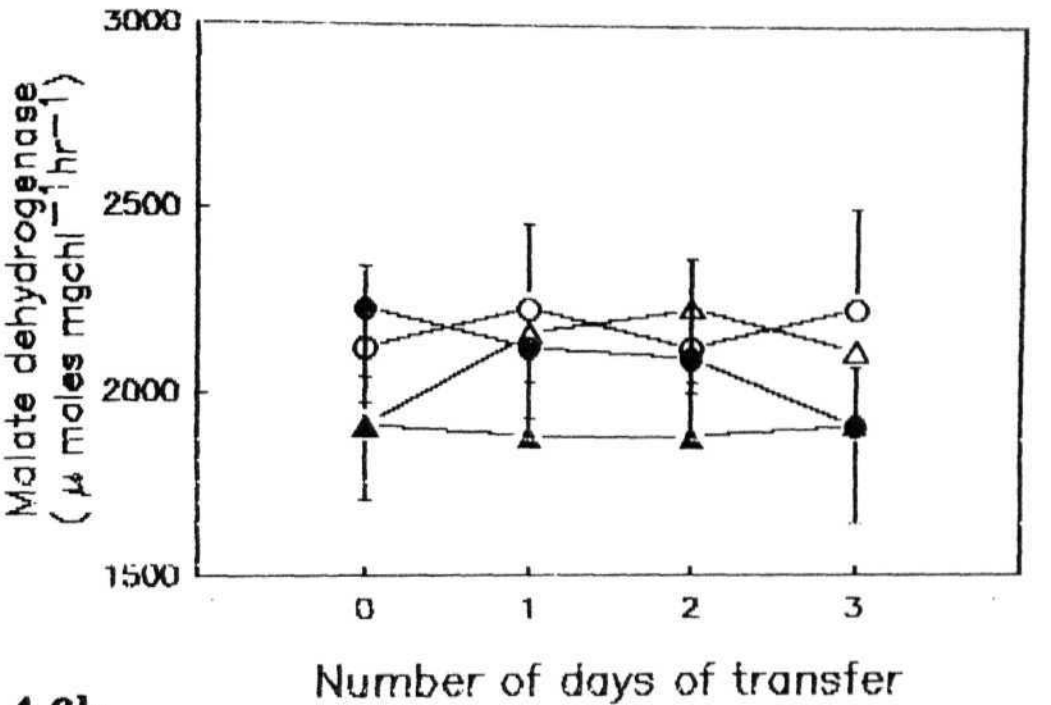
The results are average of three independent experiments.

Figure 4.6b. Effect of light intensity on malate dehydrogenase activity in *Amaranthus hypochondriacus* L.



The results are average of three independent experiments.

4.6a


Malate dehydrogenase
(μ moles mgchl⁻¹hr⁻¹)
3000
2500
2000
1500
0
1
2
3
Number of days of transfer

4.6b

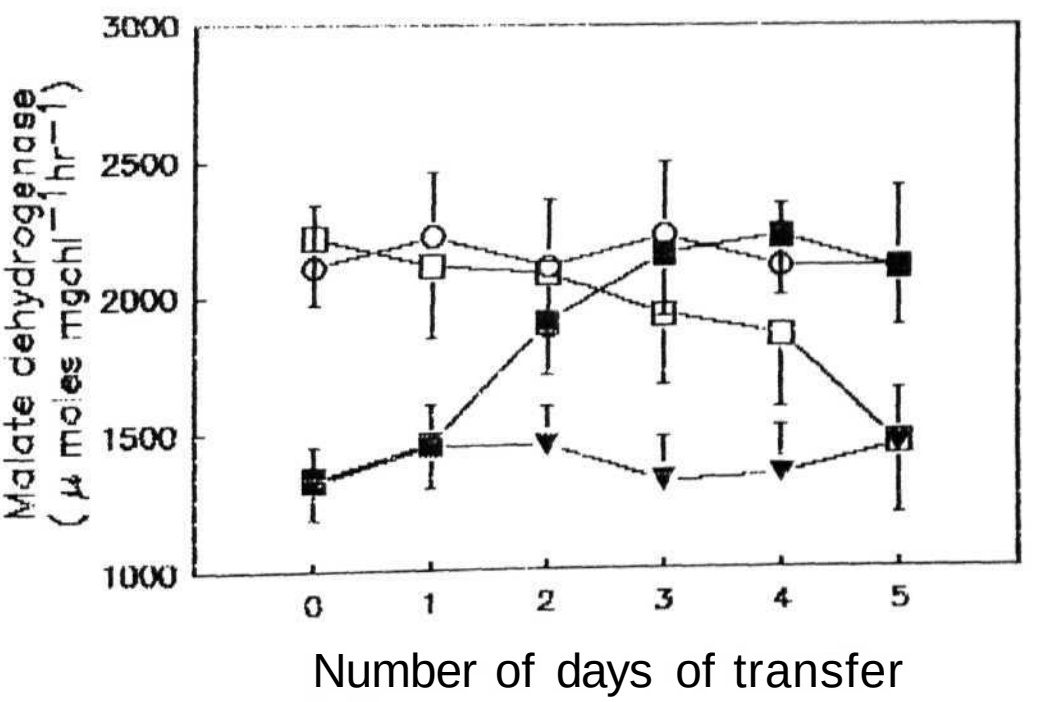
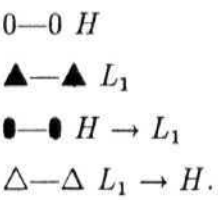
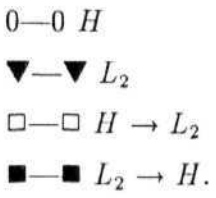

Malate dehydrogenase
(μ moles mgchl⁻¹hr⁻¹)
3000
2500
2000
1500
1000
0
1
2
3
4
5
Number of days of transfer

Figure 4.7a. Effect of light intensity on malate dehydrogenase activity in *Eleusine coracana.*



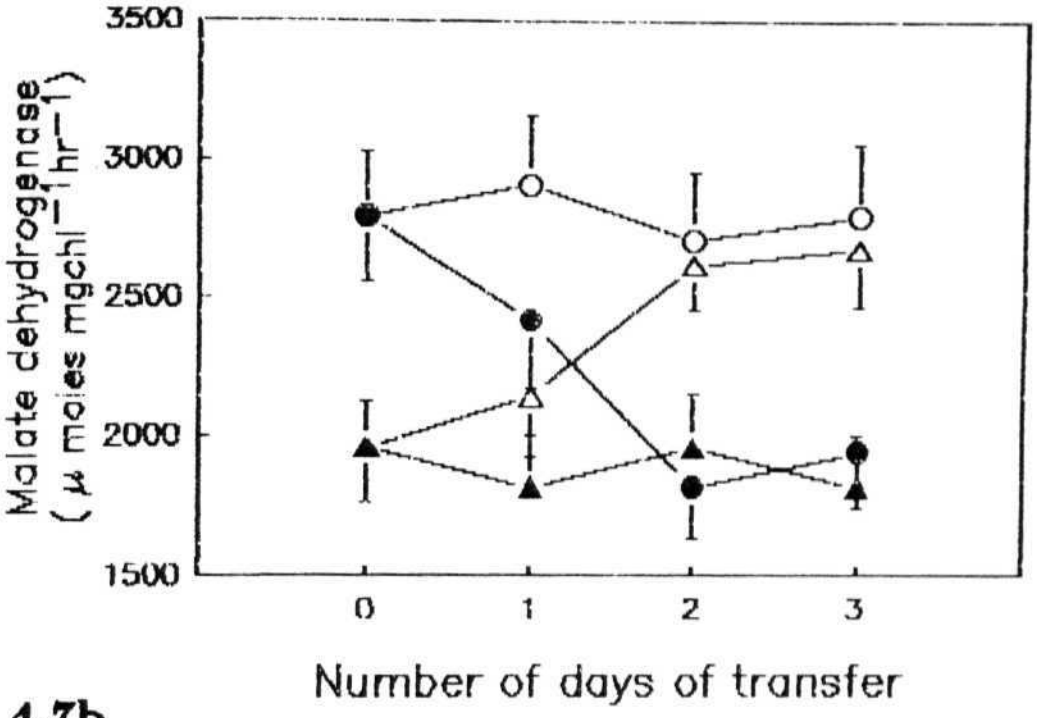
The results are average of three independent experiments.

Figure 4.7b. Effect of light intensity on malate dehydrogenase activity in *Elcusine coracana.*



The results are average of three independent experiments.

4.7a


Malate dehydrogenase
(μ moles mgchl⁻¹hr⁻¹)
3500
3000
2500
2000
1500
0
1
2
3
Number of days of transfer

4.7b

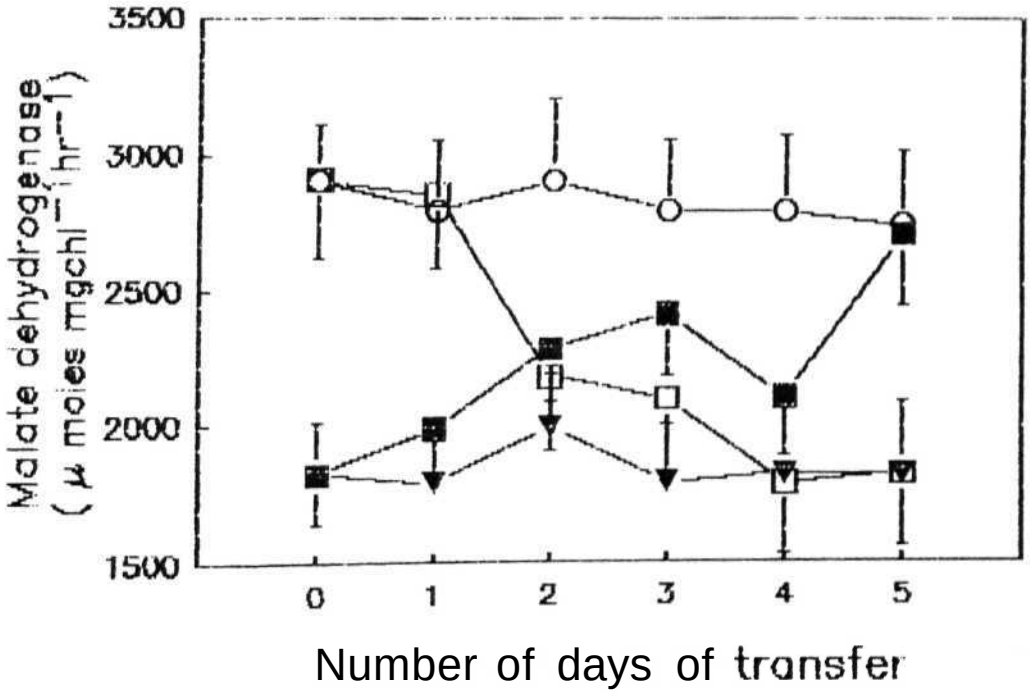
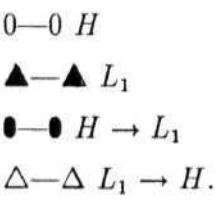
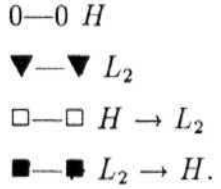

Malate dehydrogenase
(μ moles mgchl⁻¹hr⁻¹)
3500
3000
2500
2000
1500
0
1
2
3
4
5
Number of days of transfer

Figure 4.8a. Effect of light intensity on malate dehydrogenase activity in *Gomphrena globosa.*



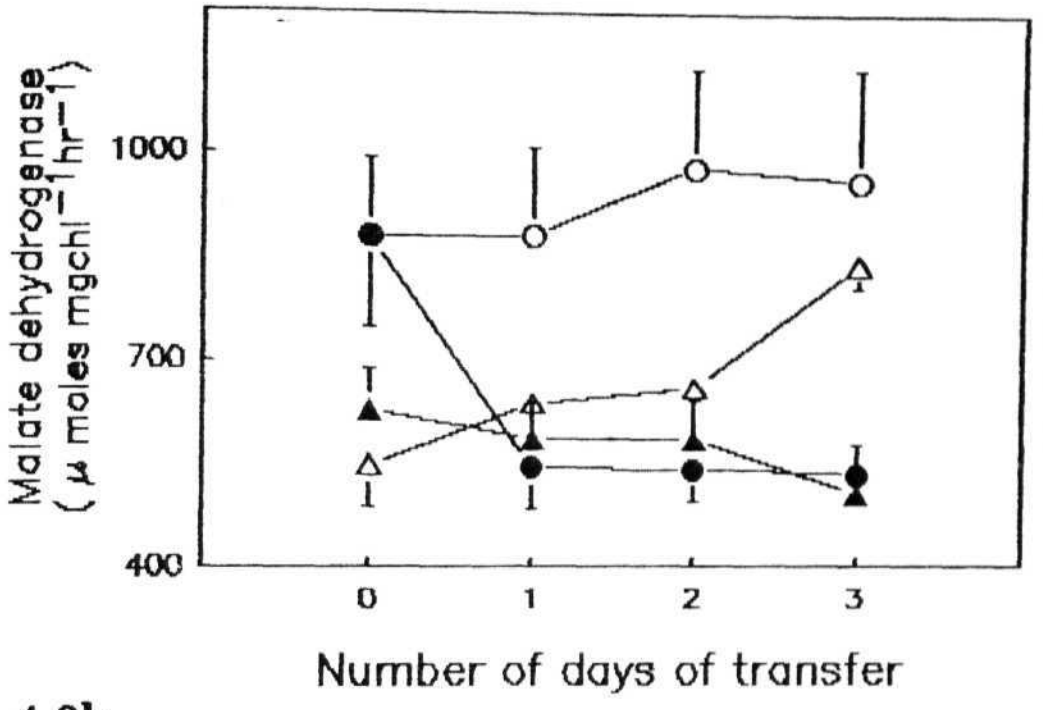
The results are average of three independent experiments.

Figure 4.8b. Effect of light intensity on malate dehydrogenase activity in *Gomphrena globosa.*

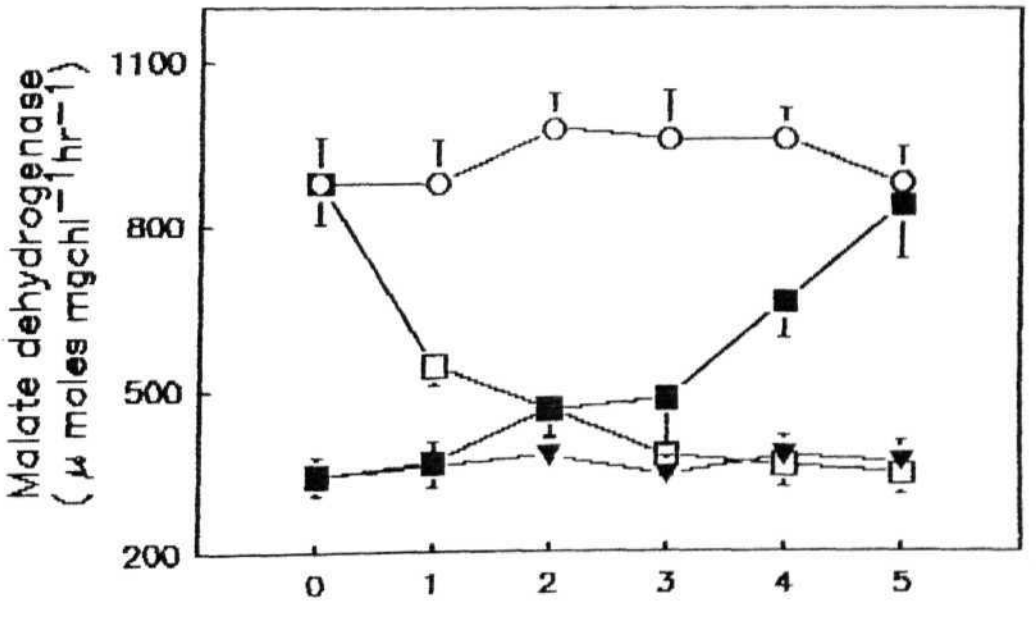


The results are average of three independent experiments.

4.8a


Malate dehydrogenase
(μ moles mgchl⁻¹hr⁻¹)
1000
700
400
0
1
2
3
Number of days of transfer

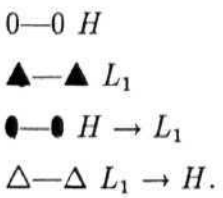
4.8b


Malate dehydrogenase
(μ moles mgchl⁻¹hr⁻¹)
1100
800
500
200
0
1
2
3
4
5
Number of days of transfer

by 77% in *Amaranthus* and 64% in *Eleusine.* The enzyme activity increased by 326% in *Amaranthus* and 175% in *Eleusine* plants when $L_1 \rightarrow H$ plants were transferred to normal irradiance (Figures 4.9a and 4.10a) in three days. The enzyme activity increased by 810% and 453% of the activities observed in L_2 plants, when $L_2 \rightarrow H$ plants were transferred to higher irradiance in *Amaranthus* and *Eleusine* respectively (Figures 4.9b and 4.10b). When $H \rightarrow L_2$ plants were transferred to lowered irradiances the enzyme activity decreased by 89% in *Amaranthus* and 82% in *Eleusine* in a duration of five days (Figures 4.9b and 4.10b). The NAD-ME enzyme activity was most sensitive to changes in irradiance compared to other C_4 enzymes. NADP-ME activity decreased in L_1 and L_2 plants of *Gomphrena* (Figures 4.11a and 4.11b) by 73% and 88% of the activity observed in *H* plants. NADP-ME activity decreased in $H \rightarrow L_1$ and $H \rightarrow L_2$ plants of *Gomphrena* by 73% and 88% of the control activity (Figures 4.11a and 4.11b). The $L_1 \rightarrow H$ and $L_2 \rightarrow H$ plants registered 283% and 746% increased enzyme activities compared to that of low light control activity (Figures 4.11a and 4.11b Table 4.3).

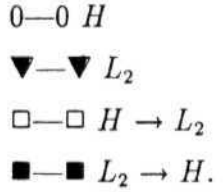
Pyruvate formed in the bundle sheath is transported to mesophyll chloroplasts where it is converted into phosphoenol pyruvate by an enzyme pyruvate orthophosphate dikinase in NADP-ME type of plant *Gomphrena globosa.* In *Amaranthus* and *Eleusine* pyruvate transport from bundle sheath to mesophyll is by reversible aminotransferase alanine aminotransferase. Alanine amino transferase activity decreased by 70%, 78% in L_1,L_2 plants of *Amaranthus* and 59%, 72% in L_1, L_2 plants of *Eleusine* respectively (Figures 4.12a, 4.12b, 4.13a and 4.13b; Tables 4.1 and 4.2). When // $\rightarrow L_1$ plants were transferred to reduced irradiance the enzyme activity decreased by 70% and 59% in *Amaranthus* and *Eleusine* respectively whereas the activity increased by 230% and 145% respectively in $L_1 \rightarrow H$ plants of *Amaranthus* and *Eleusine* in three days (Figures 4.12a and 4.13a;

Figure 4.9a. Light intensity effect on NAD-ME activity in *Amaranthus hypochondriacus* L.



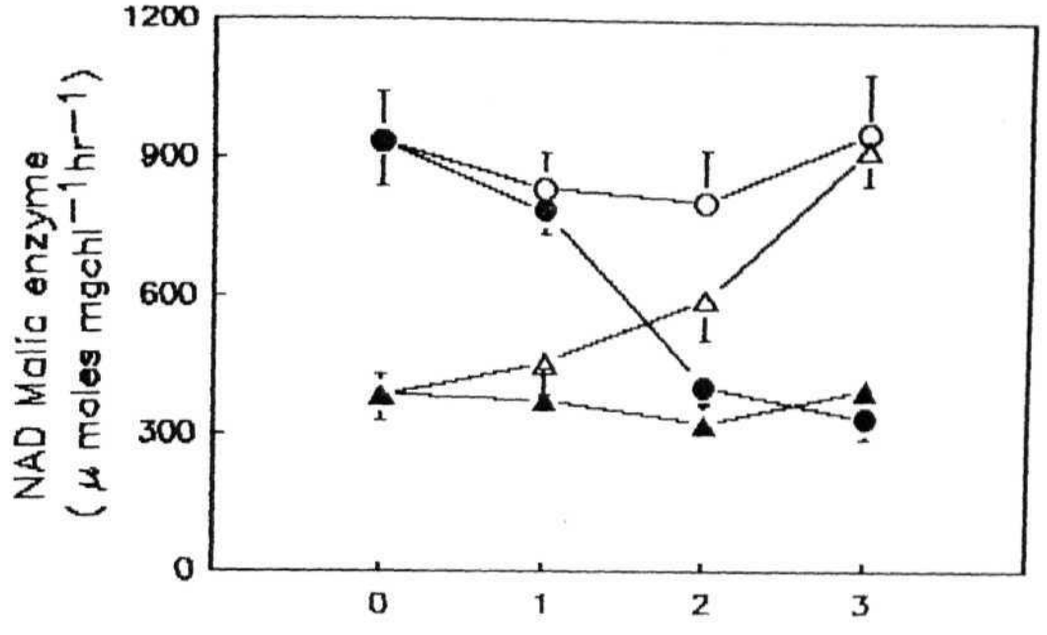
The results are average of three independent experiments.

Figure 4.9b. Light intensity effect on NAD-ME activity in *Amaranthus hypochondriacus* L.



The results are average of three independent experiments.

4.9a


1200
900
600
300
0
NAD Malic enzyme
(μ moles mgchl⁻¹hr⁻¹)
0
1
2
3
Number of days of transfer

4.9b

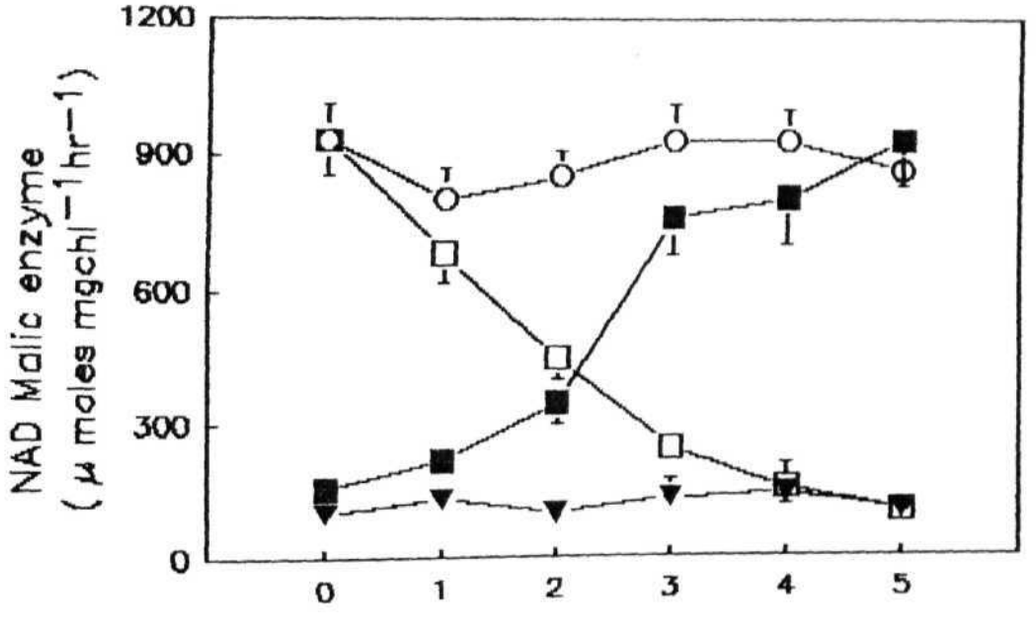
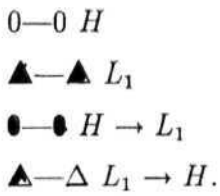
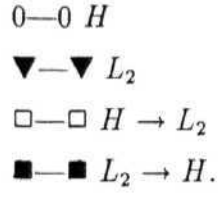

1200
900
600
300
0
NAD Malic enzyme
(μ moles mgchl⁻¹hr⁻¹)
0
1
2
3
4
5
Number of days of transfer

Figure 4.10a. Light intensity effect on NAD-ME activity in *Eleusine coracana.*



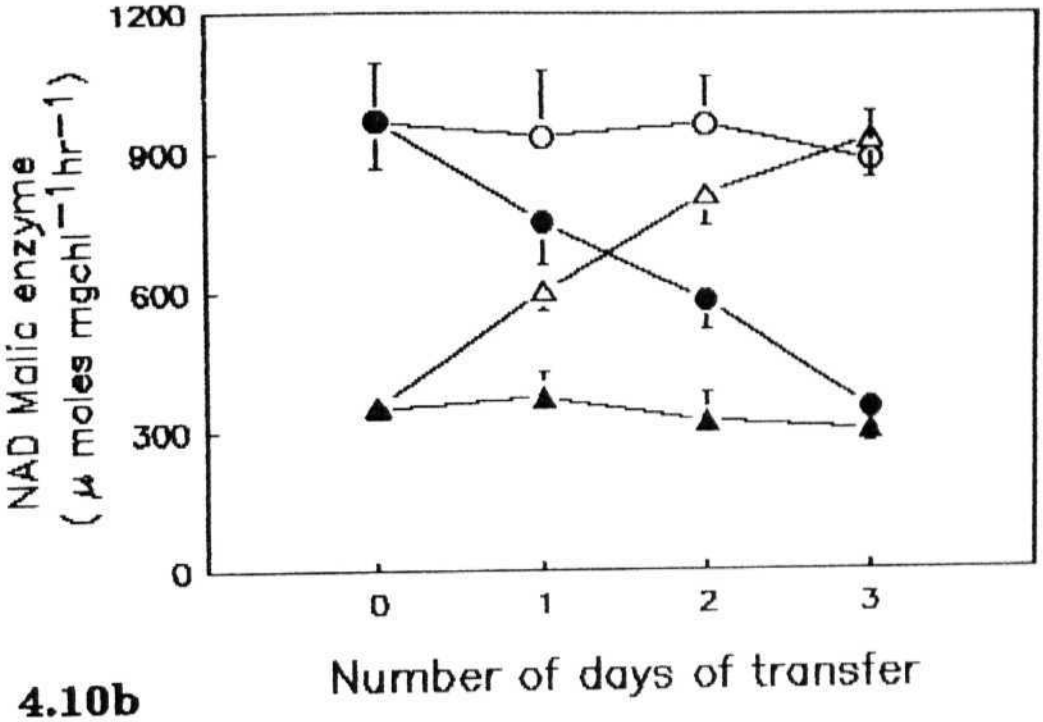
The results are average of three independent experiments.

Figure 4.10b. Light intensity effect on NAD-ME activity in *Eleusine coracana.*



The results are average of three independent experiments.

4.10a


NAD Malic enzyme
(μ moles mgchl⁻¹hr⁻¹)
1200
900
600
300
0
0
1
2
3
Number of days of transfer

4.10b

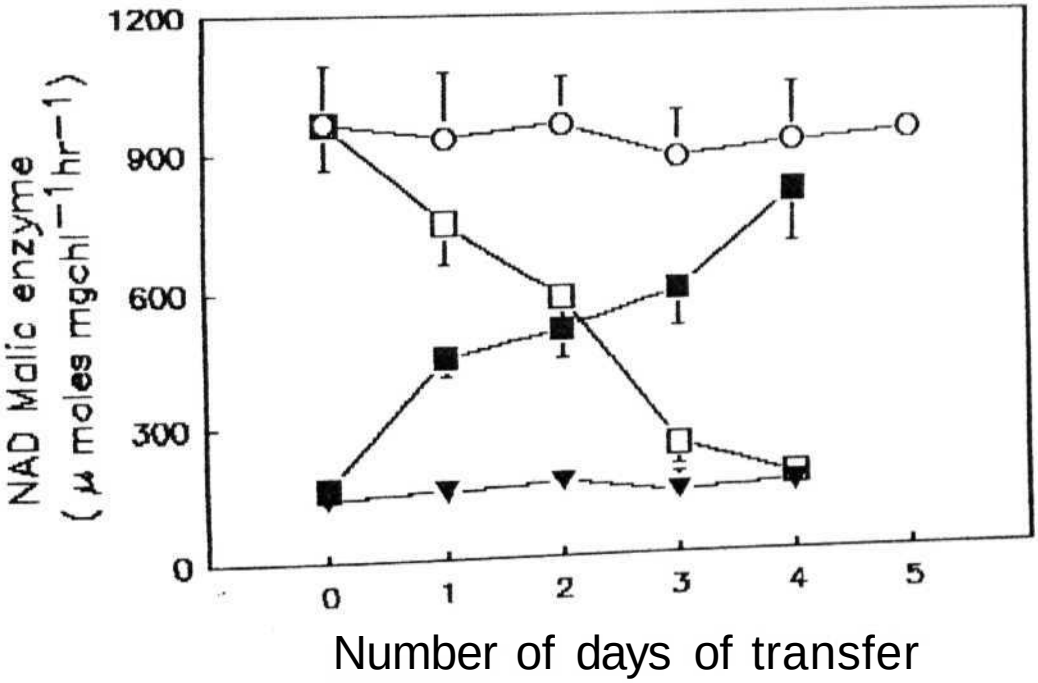
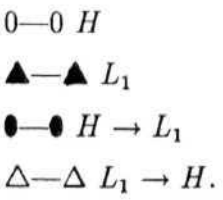
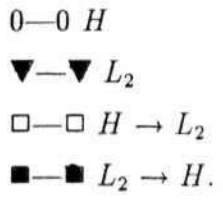

NAD Malic enzyme
(μ moles mgchl⁻¹hr⁻¹)
1200
900
600
300
0
0
1
2
3
4
5
Number of days of transfer

Figure 4.11a. Light intensity effect on NADP-ME activity in *Gomphrena globosa.*



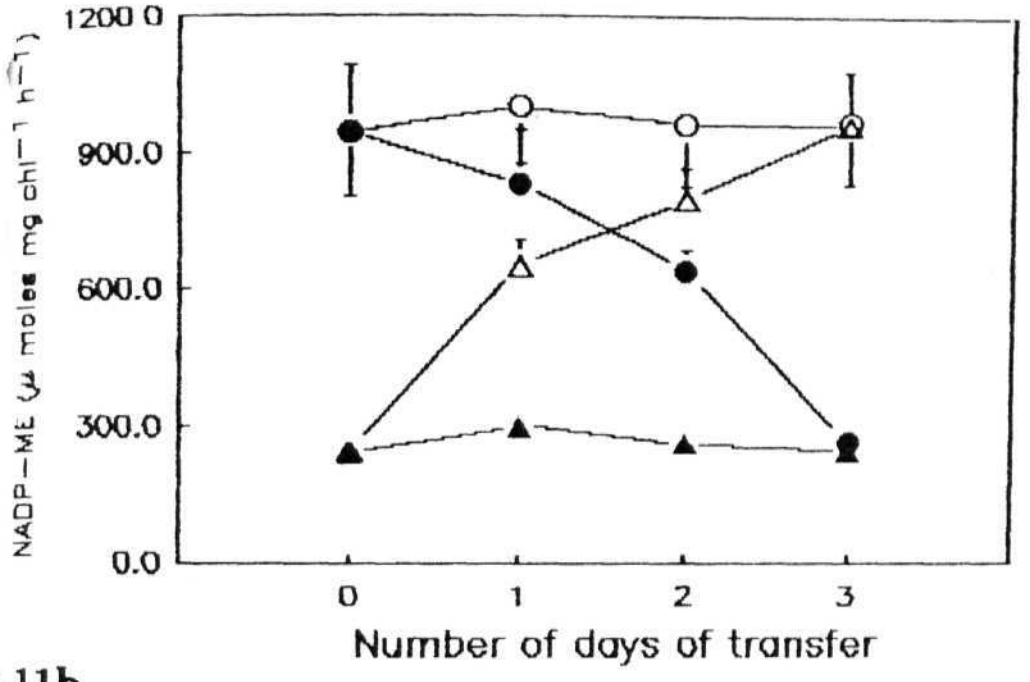
The results are average of three independent experiments.

Figure 4.11b. Light intensity effect on NADP-ME activity in *Gomphrena globosa.*



The results are average of three independent experiments.

4.11a


1200.0
900.0
600.0
300.0
0.0
NADP–ME (μ moles mg chl^{-1} h^{-1})
0
1
2
3
Number of days of transfer

4.11b

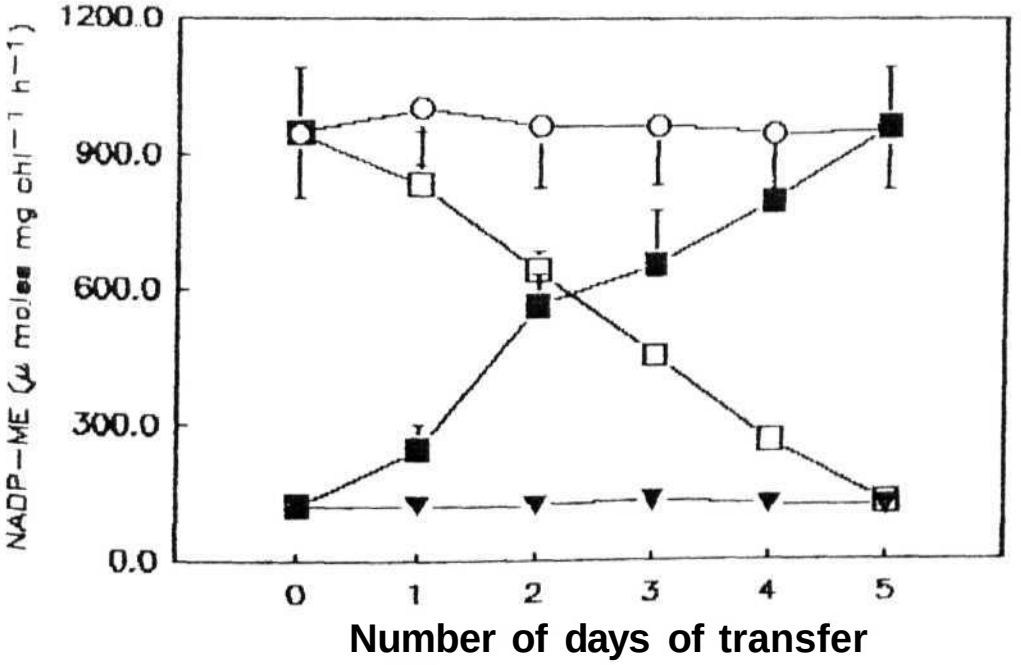
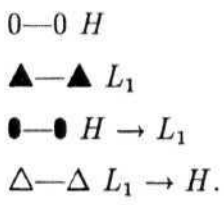
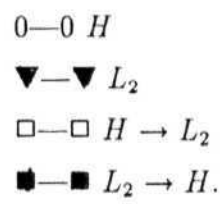

1200.0
900.0
600.0
300.0
0.0
NADP–ME (μ moles mg chl^{-1} h^{-1})
0
1
2
3
4
5
Number of days of transfer

Figure 4.12a. Light intensity effect on alanine amino transferase activity in *Amaranthus hypochondriacus* L.



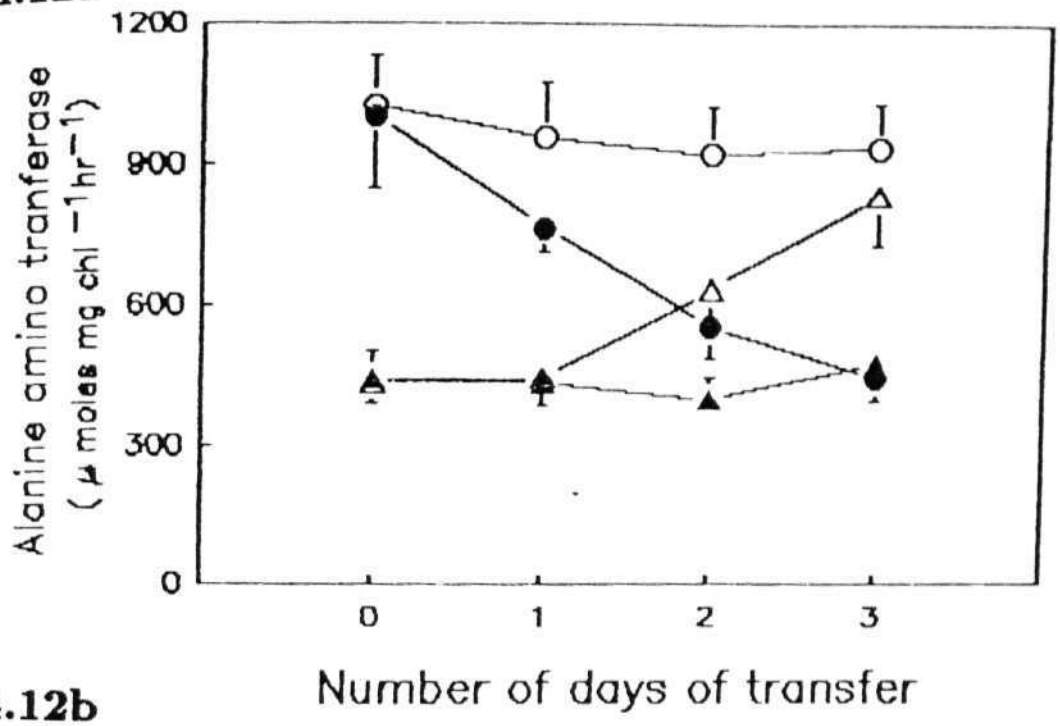
The results are average of three independent experiments.

Figure 4.12b. Light intensity effect on alanine amino transferase activity in *Amaranthus hypochondriacus.* L.



The results are average of three independent experiments.

4.12a


Alanine amino tranferase
(μ moles mg chl $^{-1}$hr^{-1})
1200
900
600
300
0
0
1
2
3
Number of days of transfer

4.12b

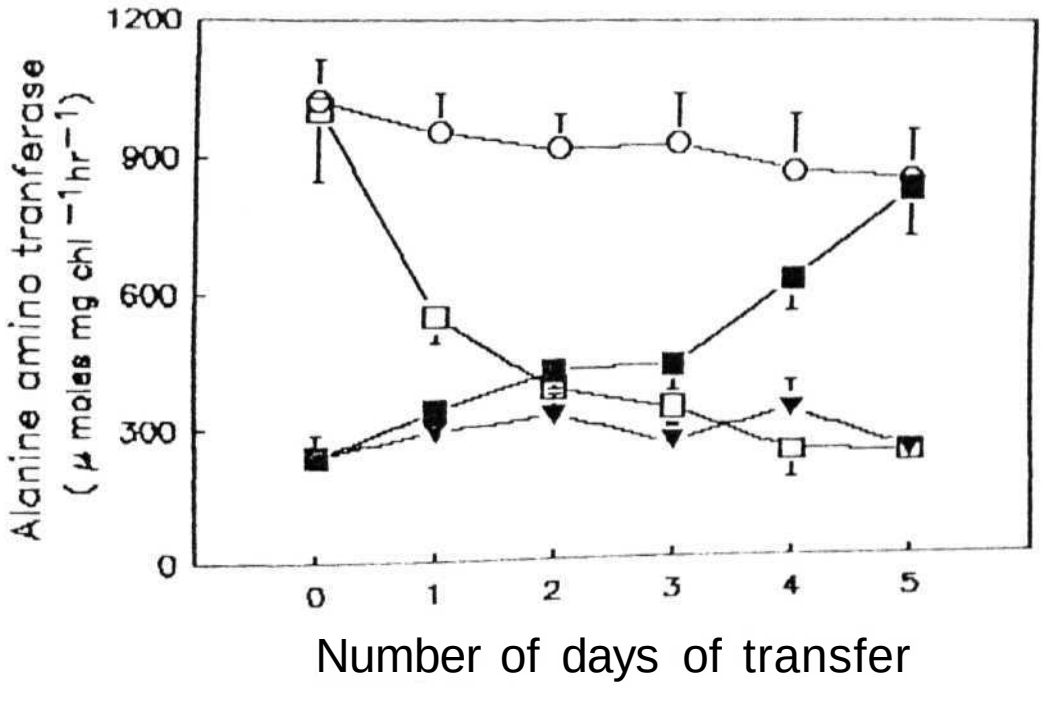
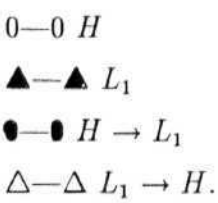
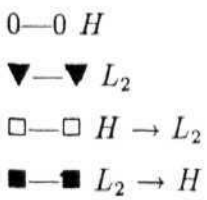

Alanine amino tranferase
(μ moles mg chl $^{-1}$hr^{-1})
1200
900
600
300
0
0
1
2
3
4
5
Number of days of transfer

Figure 4.13a. Light intensity effect on alanine amino transferase activity in *Eleusine coracana*



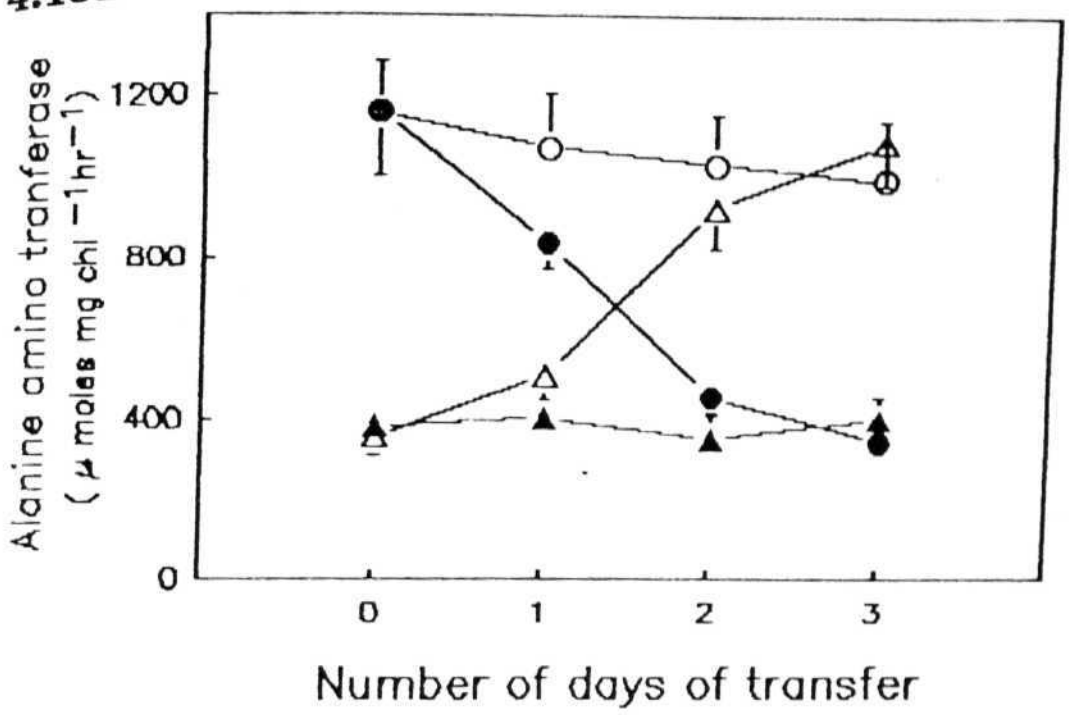
The results are average of three independent experiments.

Figure 4.13b. Light intensity effect on alanine amino transferase activity in *Eleusine coracana*

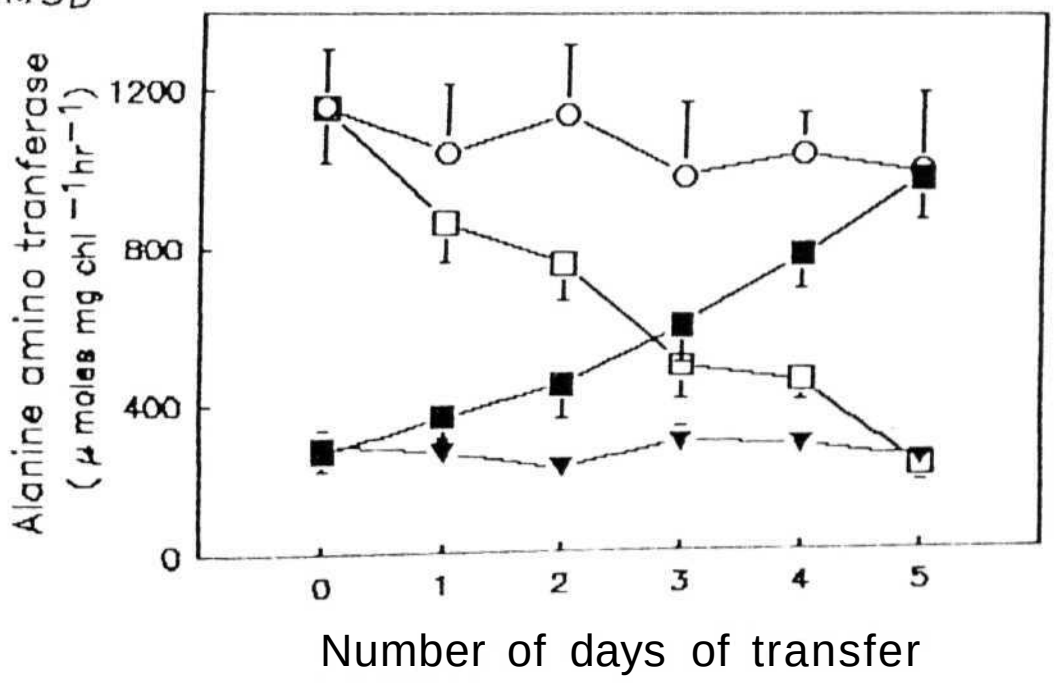


The results are average of three independent experiments.

4.13a


Alanine amino tranferase
(μ moles mg chl $^{-1}$hr^{-1})
1200
800
400
0
0
1
2
3
Number of days of transfer

4.13b


Alanine amino tranferase
(μ moles mg chl $^{-1}$hr^{-1})
1200
800
400
0
0
1
2
3
4
5
Number of days of transfer

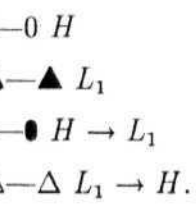
Tables 4.1 and 4.2). $H \rightarrow L_2$ plants showed 78% and 72% decline in enzyme activities in *Amaranthus* and *Eleusine* respectively whereas $L_2 \rightarrow H$ plants showed 458% and 262% increase in enzyme activity in *Amaranthus* and *Eleusine* respectively in a time period of five days (Figures 4.12b and 4.13b; Tables 4.1 and *4.2).* The alanine aminotransferase enzyme was more sensitive to the changes in growth light conditions in *Amaranthus* compared to *Eleusine.*

Pyruvate orthophosphate dikinase activity decreased by 43.8% and 69% in L_1 and L_2 plants of *Amaranthus* respectively (Figures 4.14a and 4.14b). The enzyme activity decreased in similar plants of *Eleusine* (65% and 77%) and *Gomphrena* (50% and 78%) (Figures 4.15a, 4.15b, 4.16a and 4.16b). In $H \rightarrow L_1$ plants of *Amaranthus, Eleusine* and *Gomphrena* the enzyme activity decreased by 44%, 65% and 50% respectively and $L_1 \rightarrow H$ plants showed (78%, 187% and 100%) increased activities in each of the three plants studied here, in three days (Figures 4.14a, 4.15a and 4.16a; Tables 4.1, 4.2 and 4.3). $H \rightarrow L_2$ plants on transfer to reduced irradiance showed 69%, 77% and 78% decrease in the enzyme activity in *Amaranthus, Eleusine* and *Gomphrena* plants respectively and the activity increased in $L_2 \rightarrow$ // plants by 224%, 341% and 375% respectively in a time period of five days (Figures 4.14b, 4.15b and 4.16b; Tables 4.1, 4.2 and 4.3). The percentage decrease in the enzyme activities on transfer to reduced irradiances did **not** correspond with percentage increase in enzyme activities when *Amaranthus, Eleusine* **and** *Gomphrena* plants were transferred to high irradiances.

Regulation of D_1 Protein:

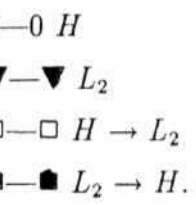
The polypeptide profile of the *Amaranthus* thylakoids grown at different light intensities is presented in Figure 5.1. It was observed that under reduced irradiances , the

Figure 4.14a. Light intensity effect on pyruvate orthophosphate dikinase activity in *Amaranthus hypochondriacus* L.



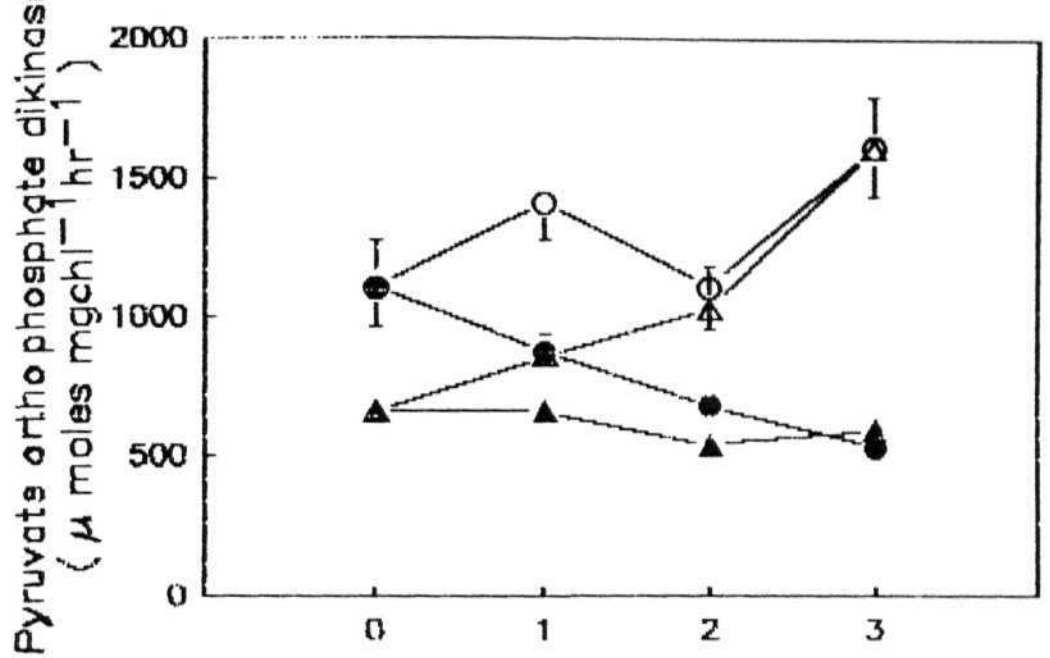
The results are average of three independent experiments.

Figure 4.14b. Light intensity effect on pyruvate orthophosphate dikinase activity in *Amaranthus hypochondriacus* L.



The results are average of three independent experiments.

4.14a


Pyruvate orthophosphate dikinase
(μ moles mgchl^{-1}hr^{-1})
2000
1500
1000
500
0
0
1
2
3
Number of days of transfer

4.14b

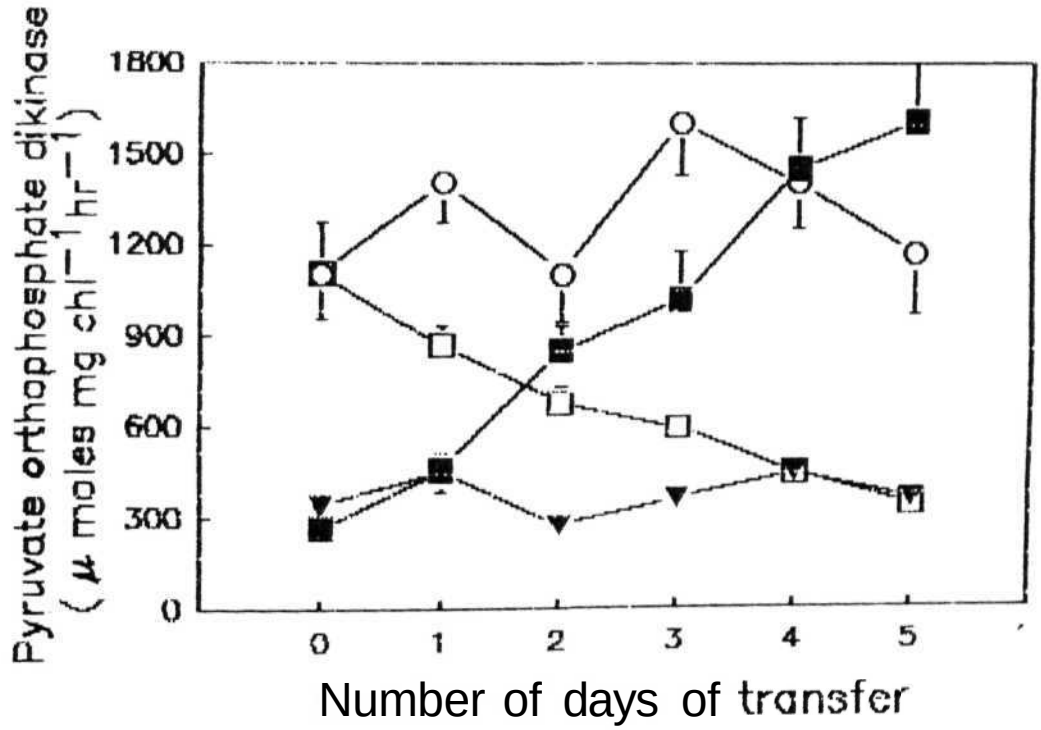
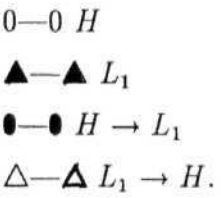
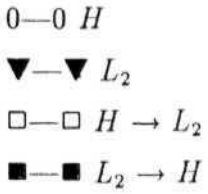

Pyruvate orthophosphate dikinase
(μ moles mg chl^{-1}hr^{-1})
1800
1500
1200
900
600
300
0
0
1
2
3
4
5
Number of days of transfer

Figure 4.15a. Light intensity effect on pyruvate orthophosphate dikinase activity in *Eleusinc coracana.*



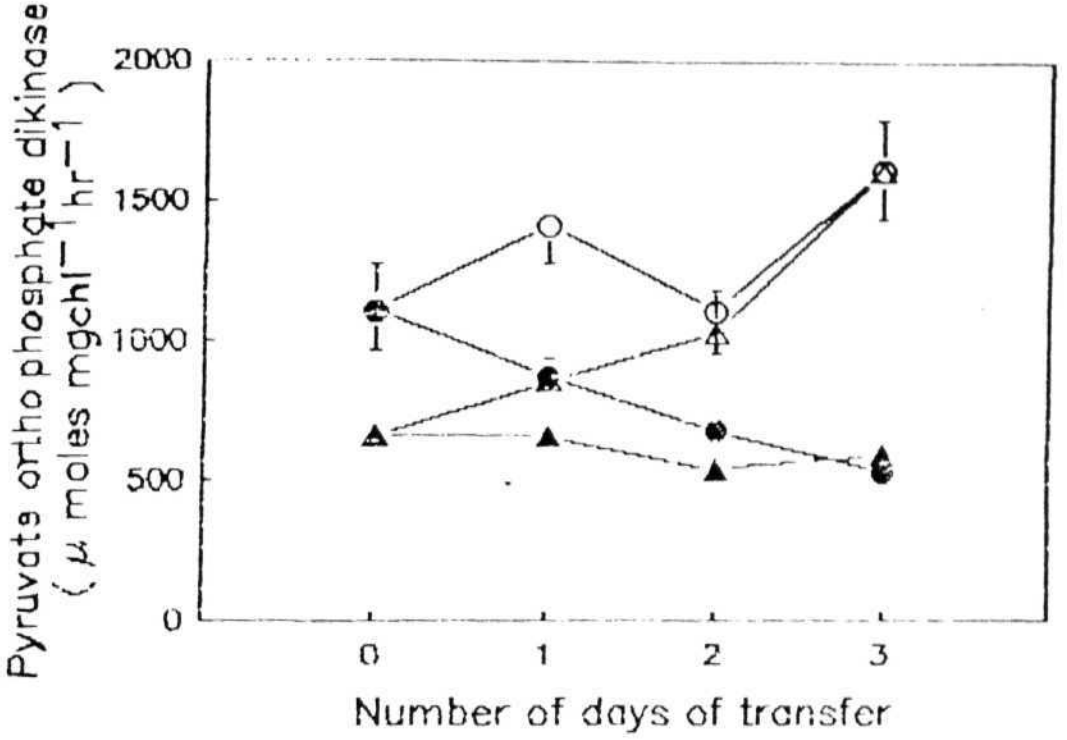
The results are average of three independent experiments.

Figure 4.15b. Light intensity effect on pyruvate orthophosphate dikinase activity in *Eleusine coracana.*



The results are average oï three independent experiments.

4.15a


Pyruvate ortho phosphate dikinase
(μ moles mgchl^{-1} hr^{-1})
2000
1500
1000
500
0
0
1
2
3
Number of days of transfer

4.15b

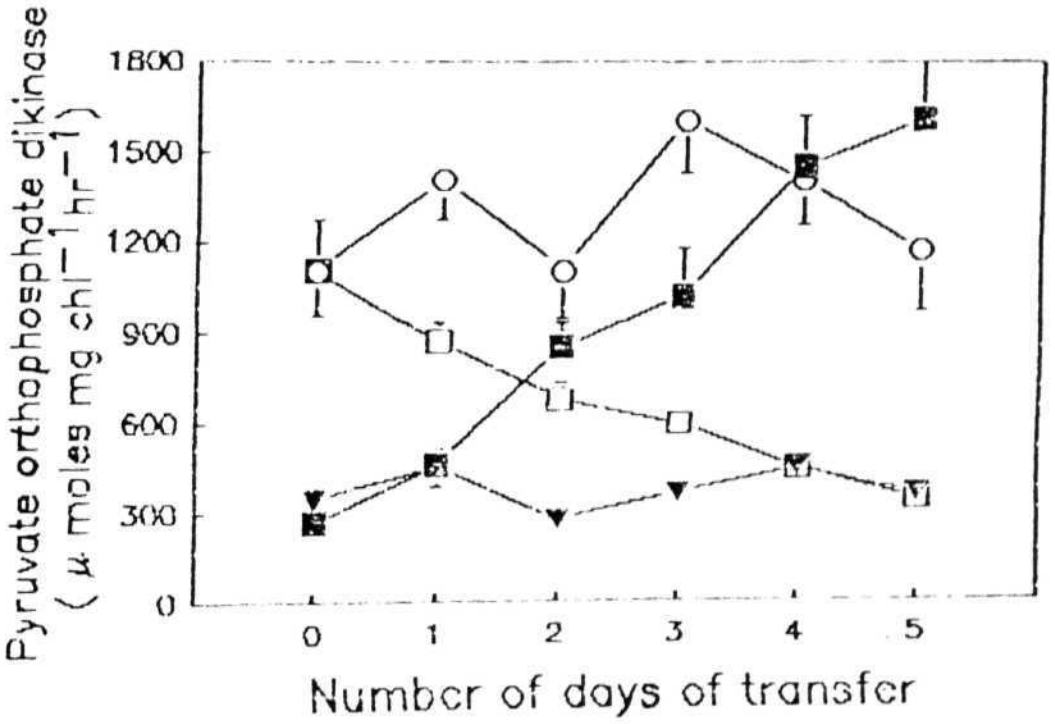
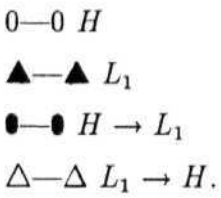
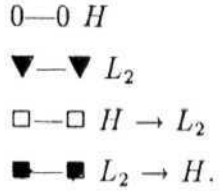

Pyruvate orthophosphate dikinase
(μ moles mg chl^{-1} hr^{-1})
1800
1500
1200
900
600
300
0
0
1
2
3
4
5
Number of days of transfer

Figure 4.16a. Light intensity effect on pyruvate orthophosphate dikinase activity in *Gomphrena globosa.*



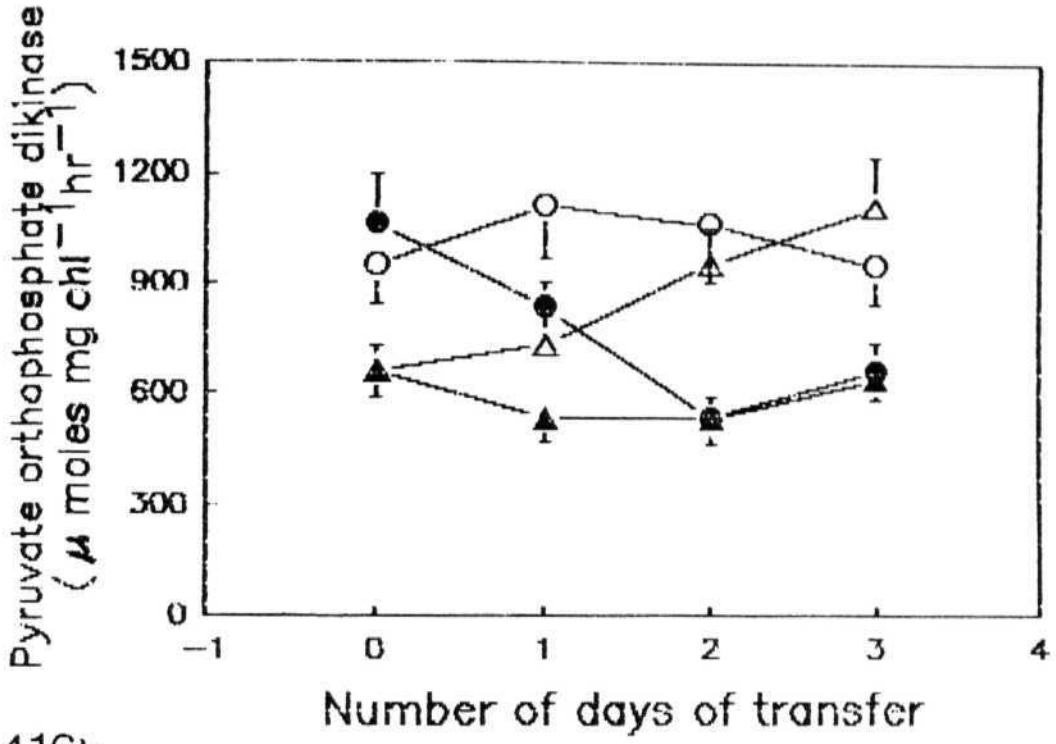
The results are average of three independent experiments.

Figure 4.16b. Light intensity effect on pyruvate orthophosphate dikinase activity in *Gomphrena globosa.*



The results are average of three independent experiments.

4.16a


Pyruvate orthophosphate dikinase
(μ moles mg chl⁻¹ hr⁻¹)
1500
1200
900
600
300
0
−1
0
1
2
3
4
Number of days of transfer

4.16b

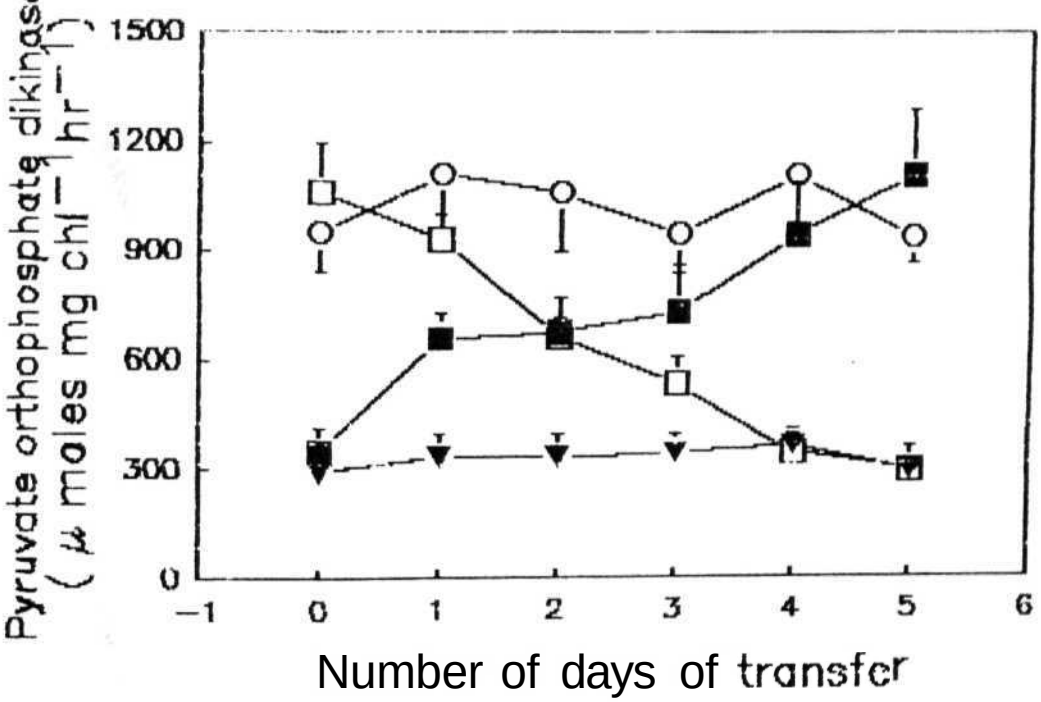

Pyruvate orthophosphate dikinase
(μ moles mg chl⁻¹ hr⁻¹)
1500
1200
900
600
300
0
−1
0
1
2
3
4
5
6
Number of days of transfer

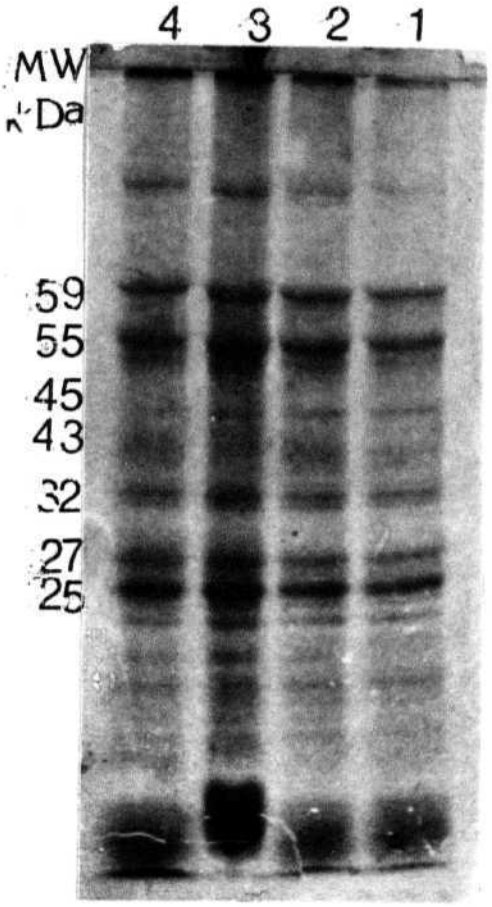
Figure 5.1. Effect of light intensity on polypeptide profile of thylakoid membranes in *Amaranthus hypochondriacus* L (SDS-PAGE).

Lane 1. Polypeptide profile of thylakoid membranes grown at normal irradiance of 2000 μE $m^{-2}s^{-1}$.

Lane 2. Polypeptide profile of thylakoid membranes grown at reduced irradiance of 650 μE $m^{-2}s^{-1}$.

Lane 3. Polypeptide profile of $H \rightarrow L_1$ thylakoid membranes.

Lane 4. Polypeptide profile of $L \rightarrow H$ thylakoid membranes.


4
3
2
1
MW
kDa
59
55
45
43
32
27
25

polypeptides associated with light harvesting complex of PSII increased (Figure 5.2b). The polypeptides comprising the ATP synthetase, the 55 kDa and 59 kDa and PSII reaction centre core polypeptides, 31 kDa and 32 kDa declined under limiting light conditions.

The $L_1 \rightarrow H$ plants on transfer to normal irradiance exhibited rise in 55 kDa, 59 kDa, 31 kDa and 32 kDa polypeptides. The polypeptides involved in the assembly of LHCII, the 27 kDa and 25 kDa decreased (Figure 5.2d). When $H \rightarrow L_1$ plants were acclimated to lowered irradiances the LHCII polypeptides increased while the polypeptides required for the assembly of ATP synthetase and PSII reaction centre decreased (Figure 5.2c). In general under suboptimal light polypeptides comprising the light harvesting complex of PSII increased at the expense of those of PSII reaction centre whereas, the reverse was true at normal irradiance.

The increase in the polypeptides of LHCII at lowered irradiance was confirmed further by scanning densitometric analysis of LHCII complex isolated from thylakoids of normal and reduced irradiance grown *Amaranthus* plants (Figure 5.3a and 5.3b). The fluorescence peak observed at 685 nm (at 774°K) also increased for *Amaranthus* plants grown at suboptimal irradiance (Figure 5.4).

The total RNA was isolated from the leaves of *Amaranthus* plants grown at different light regimes and Northern blotted to nylon membrane (materials and methods). The Northernblot was hybridized with P^{32} labelled psbA gene from *Nicotiana tabacum* (Figure 5.5). The psbA gene transcript decreased in the plants grown at lowered irradiance compared to that of normal light grown plants. The mRNA of psbA gene increased when $L_1 \rightarrow H$ plants were transferred to normal irradiance. The gene transcripts decreased

Figure 5.2. Scanning densitogram of thylakoid polypeptides isolated from plants grown under different light intensities (SDS-PAGE).

(a) Scanning densitogram of thylakoid polypeptides from plants grown at 2000 μE $m^{-2}s^{-1}$.

(b) Scanning densitogram of thylakoid polypeptides from plants grown at 650 μE **$m\text{-}V^{1}$.**

Figure : 52

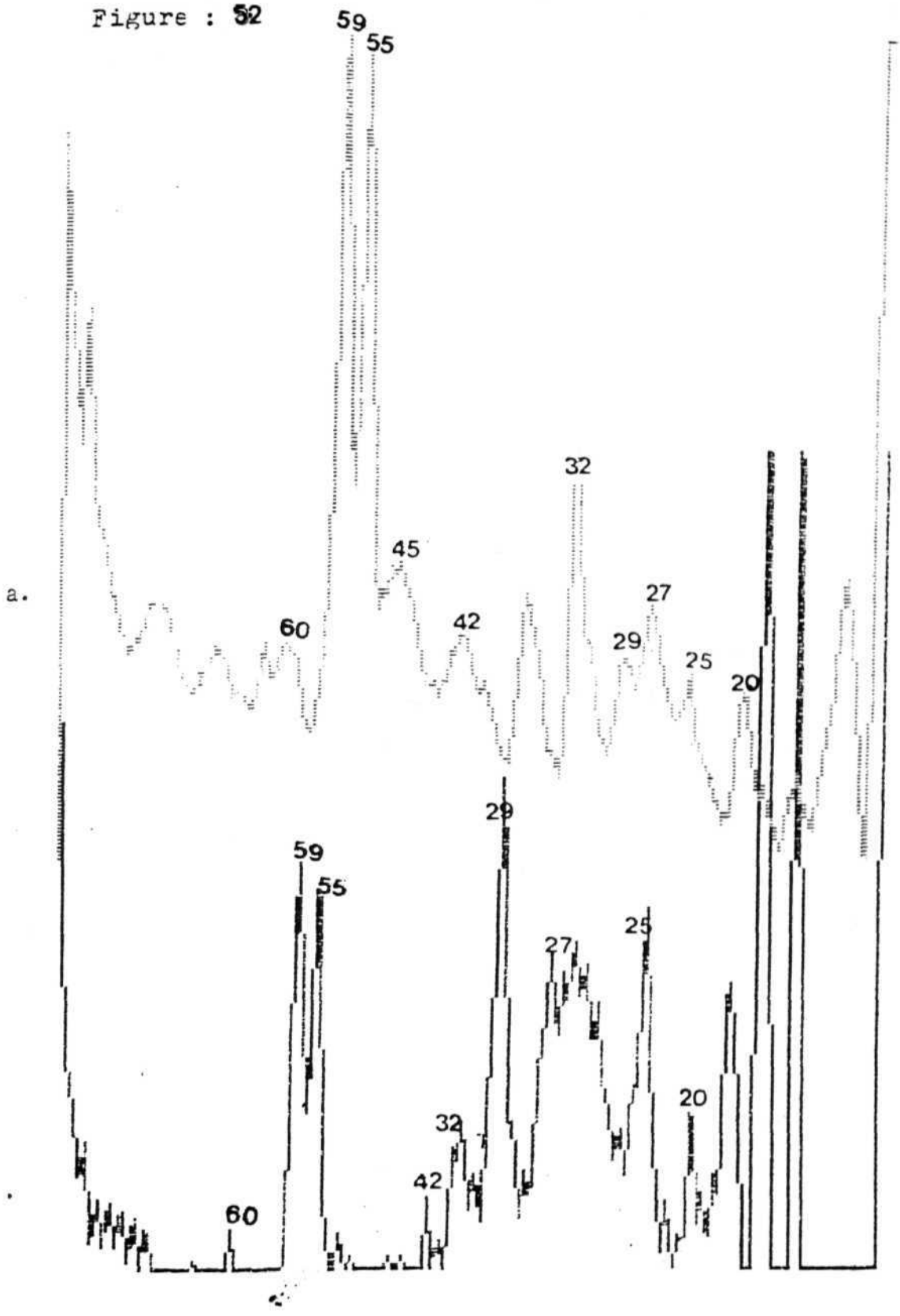


Figure 5.2. Scanning densitogram of thylakoid polypeptides isolated from plants grown under different light intensities (SDS-PAGE).

(c) Scanning densitogram of thylakoid polypeptides from $H \rightarrow L\backslash$ plants.

(d) Scanning densitogram of thylakoid polypeptides from $L_1 \rightarrow H$ plants.

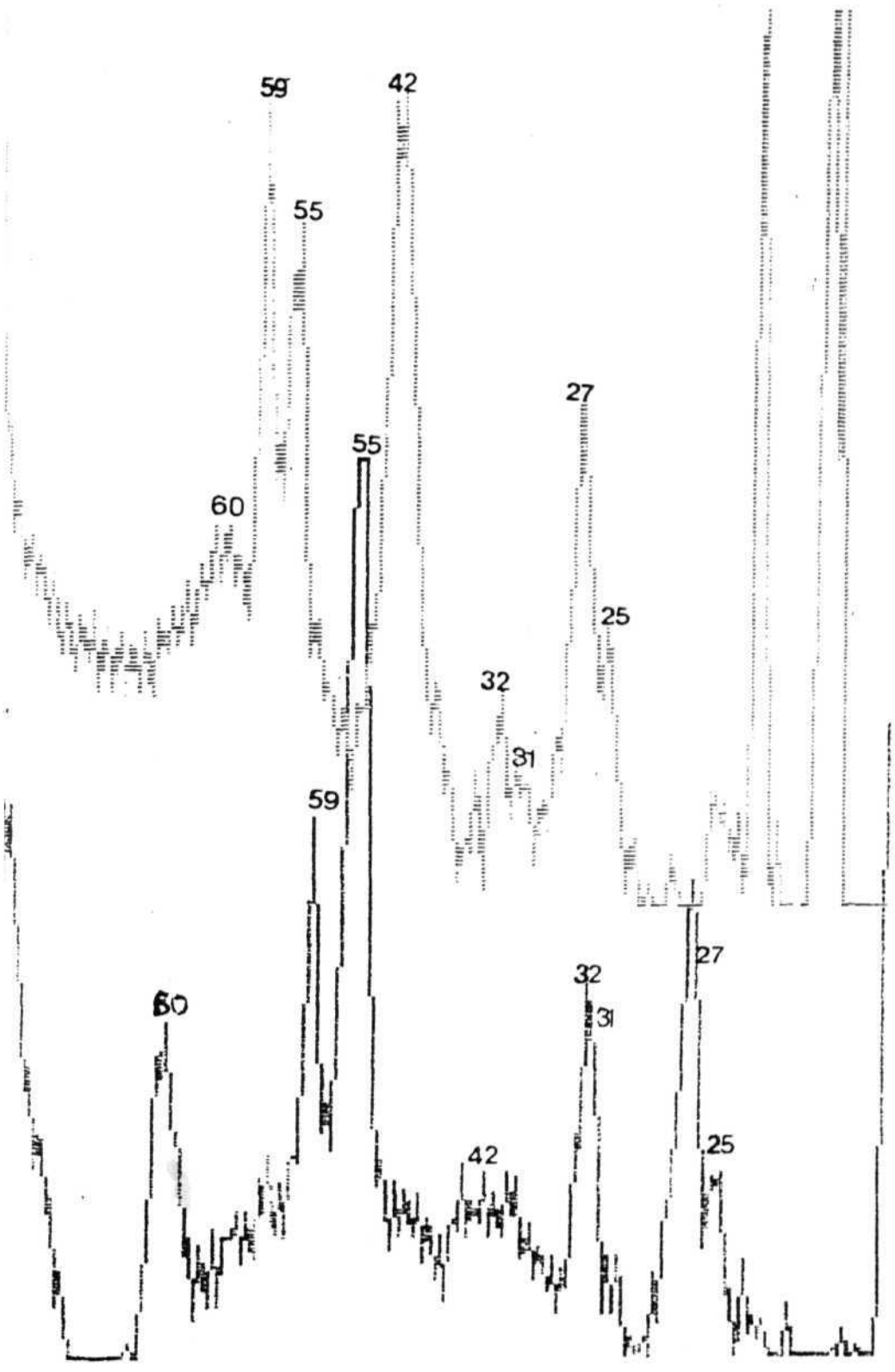

59
42
55
27
55
60
25
32
31
59
27
32
31
60
42
25

Figure 5.3a. Polypeptide profile of LHC1I complex isolated from *Amaranthus hypochondriacus.* L. grown at different light intensities.

Lane 1: LHCII from *H* plants. Lane II: LHCII from *L* plants.

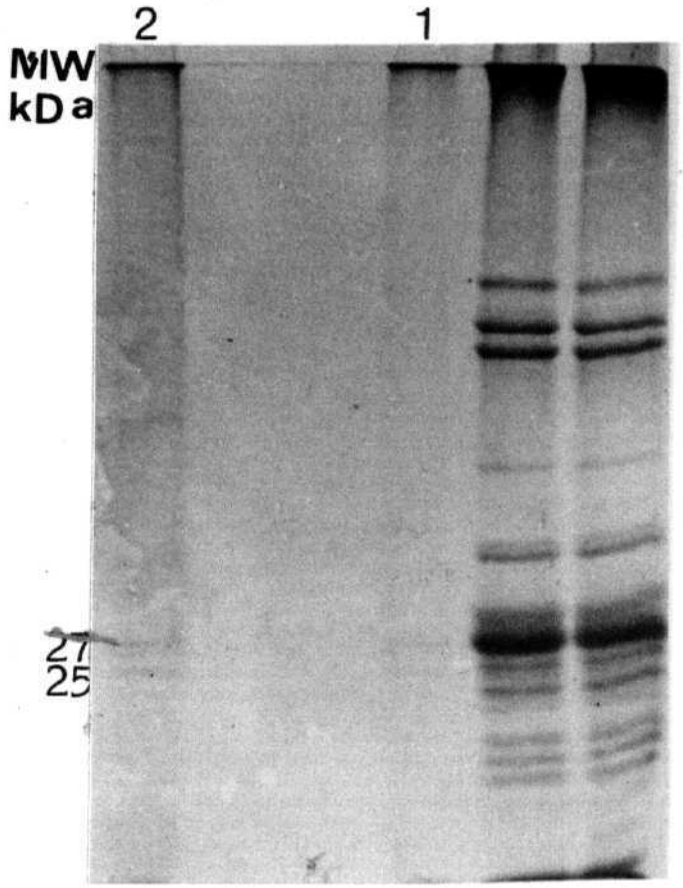
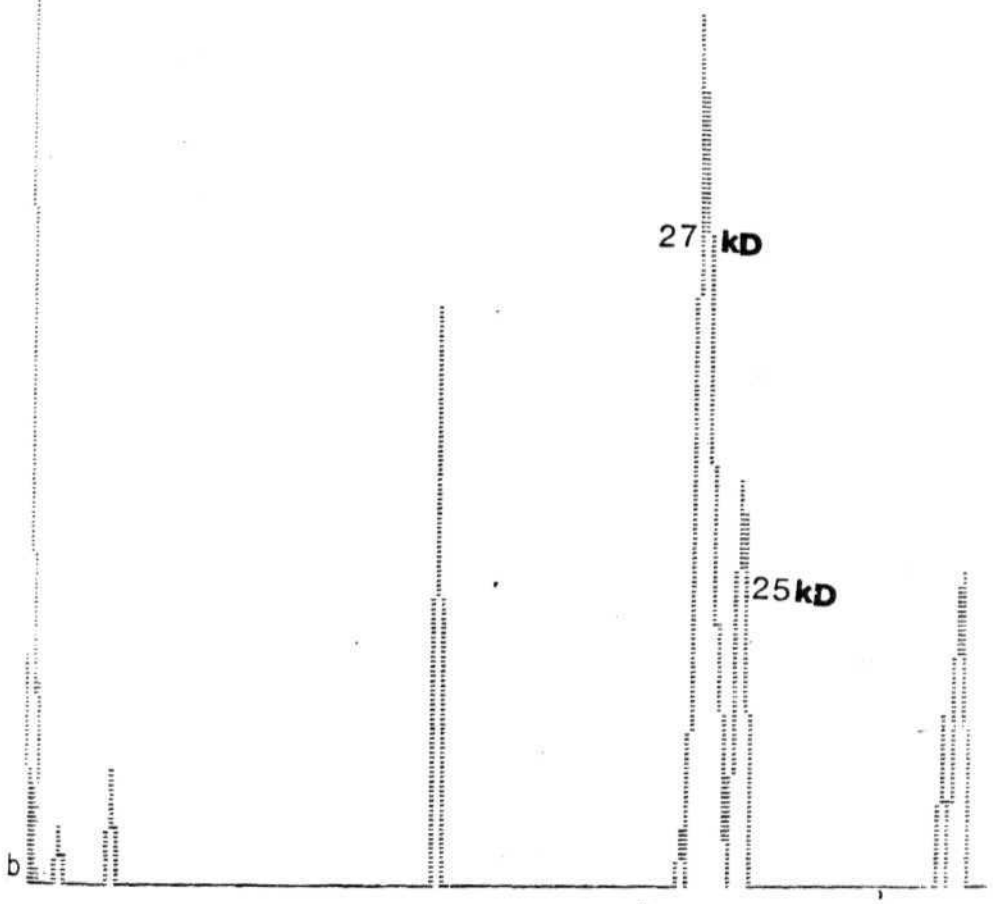

2
1
MW
kDa
27
25

Figure 5.3b. Densitogram of polypeptide profile of LHCII polypeptides isolated from *Amaranthus hypochondriacus* L grown at different light intensities.

a) LHCII from *H* plants.

b) LHCII from L_1 plants.

Figure 5.3b



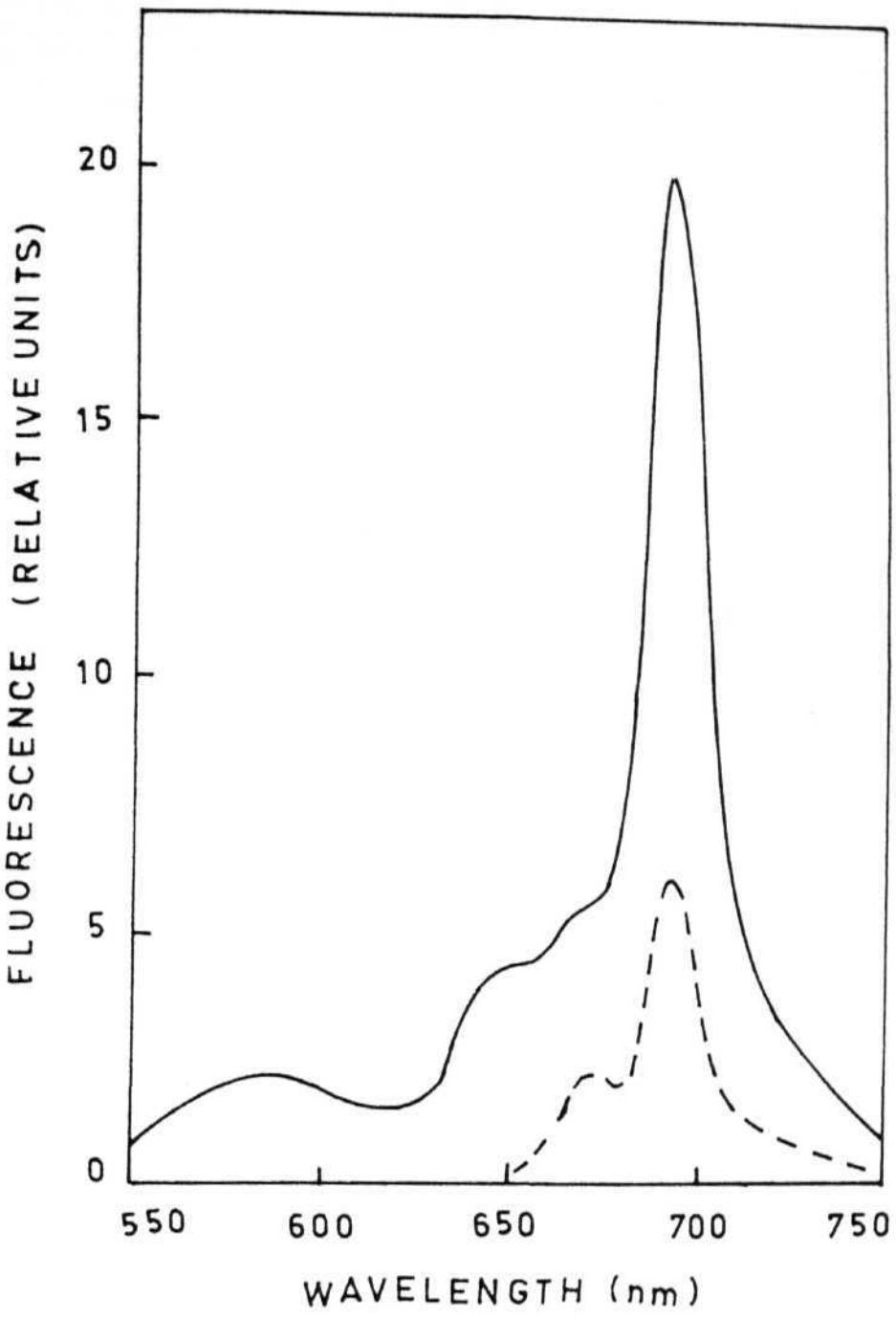


Figure 5.4: Fluorescence emission spectra of LHCII isolated from Amaranthus hypochondriacus L at 77°K.

---- Normal light grown

——— Reduced light grown

Figure 5.5. Effect of light intensity on transcript levels of psbA gene coding for *D* polypeptide in *Amaranthus hypochondriacus* L.

Lane 1. Transcript levels from leaves of $H \rightarrow L$\ plants after one day of acclimation to reduced light intensities.

Lane 2. Transcript levels from leaves of $L_1 \rightarrow H$ plants after one day of acclimation.

Lane 3. Transcript levels from leaves of L_1 plants.

Lane 4. Transcript levels from leaves of *H* plants.

Lane 5. Transcript levels from leaves of H —+ L_1 plants after 3 days of acclimation to reduced irradiance.

Lane 6. Transcript levels from leaves of $L_1 \rightarrow H$ plants after 3 days of acclimation to high irradiance.

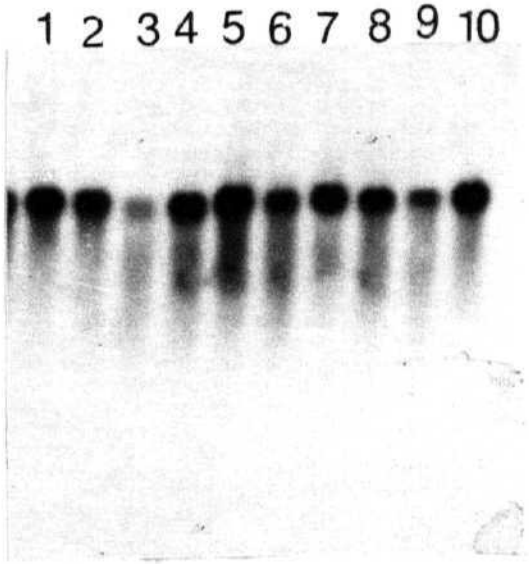
Lane 7. Transcript levels from leaves of $H \rightarrow L_1$ plants after 5 days of acclimation to reduced irradiance.

Lane 8. Transcript levels from leaves of L\ $\rightarrow$ // plants after 5 days of acclimation to high irradiance.

Lane 9. Transcript levels from leaves of H —> L_1 plants after 6 days of acclimation to high irradiance.

Lane 10. Transcript levels from leaves of L\ $\rightarrow$ *H* plants after 6 days of acclimation to high irradiance.

5 μg of total RNA was loaded in each lane and probed with psbA gene from. *Nicotiana tabacum* coding for *D* polypeptide of PSII reaction centre.


1 2 3 4 5 6 7 8 9 10

when $H \rightarrow L_1$ plants were adapted to suboptimal light. The **duration** for **the alteration** in the psbA gene transcripts was six days (Figure 5.5). The light induced increase in the psbA gene transcripts coincided with the increase in the levels **of** its **gene product** (the *D*\ polypeptide) when normal light conditions were restored (Figures 5.2d and 5.5).

DISCUSSION

PSI is known to be light-driven plastocyanin: ferridoxin oxido reductase that mediates electron transport from reduced plastocyanin to oxidized ferridoxin from the lumen side to the stroma. (Scheller and Moller 1990; Golbeck 1992). The PSI constitutes numerous polypeptides (11 to 13 depending upon species). Schantz and Bogorad (1988) named the PSI polypeptides after the genes encoding them. The PSI complex isolated from *Amaranthus* was PSI_{100} which was devoid of LHCPI (Figure 1.1). The original complex isolated earlier by others was PSI_{200} which consisted of LHCPI also (Mullet *et al.* 1980; Malkin *et al.* 1985). The observed fluorescence emission peak at 720 nm (Figure 1.2) is due to chlorophyll fluorescence from the PSI reaction centre. The EPR signal at g=1.99 arises from P_{700} chlorophyll (Miller and Brudwig 1991).

The preparation of PSI from maize lacked 18 kDa PSI-F polypeptide whereas it was present in PSI preparation from spinach. This led Nechushtai *et al.* (1986) to conclude that there is fundamental difference in the structural organization between C_3 and C_4 PSI reaction centre complexes. However Whyn *et al.* (1989) showed no such differences in the structural organization of PSI reaction centre between C_3 and C_4 plant species. From the present work on *Amaranthus hypochondriacus* L. a C_4 dicotyledonous plant the presence of 18 kDa polypeptide was noticed. Therefore from this present study it may be understood that PSI structure is conserved in both C_3 and C_4 plants. Though further analysis was not done here to find out possible alterations in the structural composition of PSI under limiting light conditions, this study has re-established the basic structure of PSI in *Amaranthus.*

The PSII reaction centre from *Amaranthus* was active for light induced silicomolybdate reduction (Figure 1.4). Earlier researchers have isolated the minimal functional unit

from cyanobacteria, C_3 and C_4 monocot plants. (Mattoo *et al.* 1986; Barber *et al.* 1987; Marder *et al.* 1987; Nanba and Satoh 1987; Shuvalov *et al.* 1989; Amrutavalli ***et al.*** 1990; Fontinou and Ghanotakis 1990; Ghirardi *et al.* 1993). The PSII reaction centre reported here is of similar composition to that isolated from various other species. The low temperature fluorescence peak at 685 nm (Figure. 1.5) may indicate cofactor chain consisting of cytochrome $(LP)/P_{680}/Ph$ (LP) Car 498 (Shuvalov *et al.* 1989). The multi-line EPR spectra for Mn at g=2 (Figure 1.6) signifies that water oxidizing Mn complex remained intact during the isolation of PSII reaction centre. It is believed that characterization of the PSI and PSII reaction centres has been done for the first time here, in the C_4 dicotyledonous plant *Amaranthus hypochondriacus'*

Chlorophyll a/b ratio decreased in the plants grown under reduced irradiances in each of the three C_4 plants, *Amaranthus, Eleusine* and *Gomphrena.* (Figures 2.4a, 2.4b, 2.5a, 2.5b, 2.6a and 2.6b). Similar reduction in chlorophyll a/b ratio was described earlier for shade plants and the plants grown under reduced irradiances (Björkman 1973; Boardman 1977; Lichtenthaler 1981; Rao and Das 1982; Chow and Anderson 1984a; Burkey and Wells 1991; Evans 1993b). In contrast the chlorophyll a/b ratio remained unchanged in response to changes in irradiance in pea, (Lee and Whitmarsh 1989) *Tradescantia* (Chow *et al.* 1991) and *Silene dioica* (Mckiernan and Baker 1991). Hence these workers were of the view that change in chlorophyll a/b ratio was not an esssential feature of the plants acclimated to lowered irradiance. From our present results it can be substantiated that acclimation of plants to reduced irradiances probably proceeds through changes in chlorophyll a/b ratio. The chlorophyll a/b ratio is thought to be an indirect measure of the composition of reaction centres to that of light harvesting antenna, since chlorophyll a is mostly associated with reaction centre complexes while chlorophyll b is present only in

the light harvesting antenna complex. A decrease in chlorophyll a/b ratio in the plants grown at reduced irradiances is interpreted as a preferential increase of light harvesting antenna complexes at the expense of reaction centre complexes. The alternative explanation could be that the reaction centre complexes decreased with minimal changes in light harvesting antenna.

These two possibilities were further examined by fluorescence emission spectra of thylakoids and the estimation of number of chlorophyll molecules which serve the PSI and PSII reaction centres. F_{690} fluorescence is emitted from PSII reaction centre and F_{735} fluorescence is emitted from light harvesting antenna of PSI. The higher F_{690}/F_{735} ratios at both room temperature and at 77^0K, in each of the three plants grown at lowered irradiance signify the preferential increase in the size of LHCII (Figures 2.7,2.8.2.9; Tables 2.1 and 2.2). A linear correlation was observed by earlier workers between the decrease in chlorophyll a/b ratio and increase in the F_{690}/F_{735} ratio at77°A'. (Terashima and Inoue 1984). The increase in the F_{690}/F_{735} ratio was proposed to be a measure of increase in the LHCII. The number of chlorophyll molecules which serve the reaction centres increased under lowered irradiances (Table 3.5). Therefore, it can be unequivocally concluded from our work that, the LHCII of the PSII complex has increased at the expense of reaction centre complex under limiting light. When each of the three species of plants grown under high irradiance were transferred to reduced irradiances, the light harvesting antenna increased for achieving efficient light capture, at limiting light conditions.

The plants grown under reduced irradiance on transfer to high irradiance showed diminished light harvesting antenna (Figures 2.13a, 2.13b, 2.14a, 2.14b, 2.15a, 2.15b, 2.16a, 2.16b, 2.17a, 2.17b, 3.17a, 3.17b, 3.18a, 3.18b, 3.22a, 3.22b, 3.23a, 3.23b, Table 3.5)

by decreased F_{690}/F_{735} (room temperature and at $77°K$) and chlorophyll/cytochrome f ratios. Plants with large light harvesting antenna are more prone to photoinhibition of photosynthesis under highlight intensities (Anderson and Andersson 1988). Accordingly, the reduced antenna size under high light conditions may protect the photosystems from possible inhibitory damage. The alteration in the LHCII in response to changes in light intensities is reported earlier for a few plant species. (Anderson 1980; Leong and Anderson 1984a; Bhaskar and Das 1987; Chow and Hope 1987; De la Torre and Burkey 1990a; Burkey and Wells 1991; Evans 1993a). The lack of adjustment of antenna size to changes in irradiance was observed for *Tradescantia* (Chow *et al.* 1991) which was found to be responsible for photobleaching of plants under high irradiance. It has been shown that the diploid *Oryza punctata* is poorly adapted or rather showed lack of adaptation, and was unable to grow in low light conditions despite greater partitioning of chlorophyll into LHCII. On the contrary the tetraploid *O.punctata* showed acclimation to low light without the adjustment of chlorophyll complexes. However, the adjustment in tetraploid *O.punctata* was due to increased PSII/PSI (Watanabe *et al.* 1993). In *Silene dioica* plants grown at reduced irradiance, the essential feature of acclimation was the ability to modify rapidly the stoichiometry of the two photosystems but not the alterations in light harvesting antenna (Mckiernan and Baker 1991). Hence there seem to exist two ways of acclimation to lowered irradiances. One mode involves the alteration in the size of LHCII while the other mechanism of acclimation is achieved through rigid LHCII complex but through dynamic stoichiometric ratios. According to present work, the adjustment to suboptimal light in *Amaranthus, Eleusine* and *Gomphrena*, seem to follow the former mode of acclimation which includes the modulation of light harvesting complex. It is believed that the larger LHCII complex efficiently harvests the available light during the acclimation of plants to suboptimal light.

The level of reduction in the chlorophyll *a/b* ratio at a given irradiance is not the same in each of the three plants studied. It is observed that the alterations in chlorophyll *a/b* and F_{690}/F_{735} ratio (room temperature and at $77^{o}K$) is maximum for *Amaranthus* and *Eleusine*, minimum for *Gomphrena* (Figures 2.4a, 2.4b, 2.5a, 2.5b, 2.6a and 2.6b). Chlorophyll/cytochrome f ratio increased more in *Amaranthus* and *Eleusine* compared to *Gomphrena* plants grown at given lowered irradiances (Table 3.5). It can be concluded that the greater partitioning of chlorophyll towards the LHCII at the expense of reaction centre is higher for *Amaranthus* and *Elcusine* compared to that in *Gomphrena*. Hence it is understood that a species specificity presumably exists in the manner of adjustment. Also the increase in LHCII is greater in bundle sheath thylakoids compared to that of mesophyll (Tables 2.1, 2.2, and 3.5). Like wise the decrease in LHCII is maximum for bundle sheath compared to that of mesophyll when normal light conditions are restored. Therefore the intensity of modulation of LHCII is not only species specific event but also a cell specific character in *Amaranthus*.

The minimal level of fluorescence F_o at 690 nm (room temperature and at $77^{0}K$) is due to emission from the antenna chlorophyll of the PSII, which occurs before the excitation energy is trapped by the reaction centres. The variable fluorescence (F_v) is directly related to the degree of reduction of the Q_A population (Butler and Kitajima 1975). Feed back mechanism of distribution of excitation energy between PSII and PSI is proposed. If PSII works faster than PSI, *QA* will be present in reduced state. When the reaction centre closes, less excitation energy is utilized in PSII and therefore *QA* is oxidized to reopen the PSII. The redox state of Q_A is adjusted for meaningful functional synchrony of photosystems. For optimum photosynthesis Q_A should be in oxidized state in order to maximize the number of functional reaction centres. F_m represents the condition when

all the reaction centres are closed.

The increase in F_o under lowered irradiances observed in each of the three plants studied could be due to some of the reaction centres remaining closed which are inefficient to perform photochemical work (Tables 2.3 and 2.4). Therefore F_m is lowered in these conditions. In the plants studied here, the increase in closed reaction centres under lowered irradiances is directly proportional to available growth light. At fixed reduced growth light levels the number of closed reaction centres is species specific. In limiting growth light *Gomphrena* exhibited more open reaction centres compared to that of other two plants (Tables 2.3 and 2.4). Under suboptimal light conditions due to increase in closed reaction centres, Q_A remains in greater reduced state, which results in inefficient working of PSII. The inefficient working of PSII in turn reduces the quantum efficiency of photosynthesis. The decrease in electron transport from *QA* lowered the excitation energy transfer between PSII and PSI. Thus the co-ordination between the two photosystems is adjusted in accordance with the less available light energies. The ratio F_v/F_m denotes the excitation energy captured by open reaction centres, in the absence of photochemical quenching (Genty *et al.* 1989). The decrease observed in F_v/F_m in the plants grown under limiting light signifies decrease in the excitation energy capture by the open reaction centres. Therefore, from this study, it is concluded that, under suboptimal light conditions there is impairment in the functional open PSII reaction centres because of the lowered excitation energy capture. The light harvesting antenna increased for optimizing the working of open reaction centres to negate the decrease in the excitation energy capture.

Gomphrena exhibited relatively less decline in F_v/F_m compared to that in *Amaranthus* and *Eleusine* both at room temperature and at 77^0K in response to reduction in light energies (Tables 2.3 and 2.4). The fluorescence measurements at 77^0K are more reliable than such measurements at room temperature due to lack of effect of metabolic reactions on fluorescence at 77^0K (Ogren and Öquist 1984). The less deviation in the ratio implies that the efficiency of excitation energy capture by open reaction centres is presumably higher for *Gomphrena.* Therefore the increase in the cross sectional area of the light harvesting complex (indicated earlier by least changes in F_{690}/F_{735}, chlorophyll a/6, chlorophyll/cytochrome f) at lowered irradiance is least in *Gomphrena* plants compared to that in *Eleusine* and *Amaranthus.* The assemblage of light harvesting complex is apparently larger in *Amaranthus* and *Eleusine* grown at limiting light intensities to offset the decrease in the efficiency of excitation energy capture by the open reaction centres.

The F_v/F_m ratio is susceptible to stresses of environment such as, excess light, (Björkman 1987; Adams *et al.* 1990; Krause et al. 1990) chilling temperature (Öquist *et al.* 1987; Richard and Hall 1987) and high temperature (Greer and Laining 1989; Ferguson and Burke 1991; Sharma and Hall 1992; Ollander *et al.* 1993). The F_v/F_m ratio decreased under stress conditions and was restored when the optimal conditions were re-established (Ferguson and Burkey 1991). An increase in the ratio was observed in shaded habitat for a few C_3 and C_4 plants. (Björkman and Demmig 1987). The increase in F_v/F_m ratio was seen earlier as adjustment to shade habitat for improving the efficiency of energy transfer mechanisms in suboptimal light (Björkman and Demmig 1987). *Silene dioica* plants grown at lowered irradiance did not show modulation in the open reaction centres and in the magnitude of excitation energy capture by these reaction centres (Mckiernan and Baker 1991). However, in contrast to earlier observations,

the F_v/F_m ratio was found to have decreased in the present study, when the plants were grown in suboptimal light conditions. The decline in the ratio may therefore signify a stress response rather than an adaptive measure, in each of the three plants studied. The adjustment for this response is brought about by the the augmentation of LHCII in such conditions to optimize performance at the less available light energies.

Gomphrena plants grown under limiting light exhibited less stressful response compared to other two plant species studied (Tables 2.1, 2.2, 2.3, 2.4). The alteration in F_v/F_m ratio indicates the effect of stressful environmental conditions on the process of photosynthesis. The species, more efficient for suboptimal growth conditions, can be identified quickly from least alterations in F_v/F_m under disturbed conditions. Our study identified *Gomphrena* to be more shade tolerant compared to *Amaranthus* and *Eleusine.* The factors governing such behaviour are beyond the scope of the present work.

In the plants acclimating to limiting light the number of closed reaction centres increased along with concomitant decrease in excitation energy transfer in the open reaction centres. (Figures 2.21a, 2.21b, 2.22a, 2.22b, 2.23a, 2.23b, 2.24a, 2.24b, 2.25a, 2.25b, 2.26a, 2.26b). The Q_A oxidation is modulated to regulate the excitation energy transfer between PSII and PSI. The impairment in the function of PSII reaction centre under stressful environmental conditions appeared to be a temporary phenomenon because an exposure to normal light levels restored the functional capacity of PSII . The number of open reaction centres increased enabling the greater oxidation of *QA* (Figures 2.21a to 2.26b), which facilitates the rise in excitation energy transfer in the open reaction centres and the energy transfer between PSII and PSI. The duration of time for the loss and restoration of PSII function due to the changes in light intensity is characteristic for

a given plant studied here. The time course of acclimation to one third of normal light in *Amaranthus, Eleusine* and *Gomphrena* was 6, 4 and 8 days respectively. Again, an acclimation to one tenth light was accomplished in a time period of 6, 8 and 10 days in these plants respectively. The completion of acclimation process is documented by the fact that the plants transferred to new light regimes exhibited the characters of control plants grown at that fixed irradiance. The regulation of PSII composition and function, under varying light intensities, proceeded with an initial lag of 24-48 hours in *Eleusine* and *Gomphrena*. Such initial lag was also seen during the acclimation of lucerne canopy to reduced irradiances (Evans 1993a and Evans 1993b). The factors governing such lag are presently unknown.

From the present work it can be established that the modulation of PSII structure and function in response to variations in the light intensity, is a relatively long term process obviously involving alterations in structural organization mediated through light regulated protein synthesis. Accordingly, these results are different from the rapid changes exhibited under photoinhibition.

The quantum yield of non cyclic electron transport is directly proportional to the product of q_Q (photochemical quenching) and the efficiency of excitation energy capture by open reaction centres (designated as F_v/F_m)(Genty *et al.* 1989). The q_Q in turn depends on open reaction centres. It is found that in each of the three plants under limiting light intensities the number of open PSII reaction centres and the efficiency of energy capture by these reaction centres declined (Tables 2.3 and 2.4). Therefore under lowered irradiances, decreased electron transport rates are expected. Studies with a few number of species indicated that photophosphorylation and photosynthetic electron

transport rates are curtailed in suboptimal light conditions. (Björkman 1973; Boardman 1977; Davies *et al.* 1986; Leong and Anderson 1987a; Chow and Anderson 1987b; Anderson and Andersson 1988; De la Torre and Burkey 1990b; Evans 1993a). The percentage decrease in whole chain electron transport rates due to the changes in light intensities varied in the plants studied here (Table 3.1). *Gomphrena* showed lowest reduction in the whole chain electron transport at a given lowered irradiance compared to *Amaranthus* and *Eleusine*. In other studies with limiting light levels, 40-50% variations in whole chain electron transport of pea (Leong and Anderson 1984b), four fold alteration in *Atriplex* (Boardman 1977) and two fold change in barley (De la Torre and Burkey 1990b) were noticed. In the present study too, a variation in the reduction of electron transport was noticed. The magnitude of modulation in the electron transport in response to varying light intensity seems to be a characteristic of individual plant species. Mesophyll and bundle sheath thylakoid membranes of *Amaranthus* differed in the extent of modulation in the electron transport at reduced irradiances. Hence, the magnitude of electron transport reduction is not only species specific but also cell specific.

The whole chain electron transport decreased in the plants studied here (Table 3.1; Figures 3.1a, 3.1b, 3.2a, 3.2b, 3.7a, 3.7b, 3.10a and 3.10b) when they were acclimated to limiting light intensities. The lowered electron transport is due to alterations in the composition of supra molecular complexes of thylakoid membrane. The transport is restored when the plants grown under suboptimal light conditions are re-acclimated to normal irradiance (Table 3.1; Figures 3.1a, 3.1b, 3.2a, 3.2b, 3.7a, 3.7b, 3.10a and 3.10b). The electron transport recovered to normalcy as the function of supra molecular complexes and the redox electron carriers are reinstated due to elevated light energy provided to the plants. (Tables 3.1 and 3.4)

The PSI and PSII electron transport were sensitive to reduced light (Tables 3.2 and 3.3). Mesophyll thylakoids were more susceptible to changes in irradiance compared to those of bundle sheath (Tables 3.2 and 3.3), since the magnitude of change in both PSI and PSII electron transport was higher in mesophyll. Under limiting light the impairment of PSII electron transport was higher compared to PSI. The rate limiting step is apparently PSII electron transport in the whole chain electron transport. The lowering of the PSII electron transport is due to curtailment in PSII functional reaction centres (Tables 2.3 and 2.4). Earlier, the decrease in PSII reaction centres per unit chlorophyll was also observed in other plants (Leong and Anderson 1984b; Wild *et al.* 1986; Evans 1987b; De la Torre and Burkey 1990b). The functional PSII reaction centres were increased, thus elevating the PSII electron transport when optimum light was restored. (Tables 2.3, 2.4 and 3.3). The site of regulation by variation in light intensity could be at the accepter site of PSII reaction centre involving plastoquinone pool. For a given change in light intensity the alteration in the PSII electron transport was found to be least in *Gomphrena* (Table 3.3). It was observed earlier that the variations in number of open reaction centres and energy captured by the open reaction centres were least in *Gomphrena*. The alteration in Q_A reduction was also found to be least compared to that of other two plants at lowered irradiances (Tables 2.3 and 2.4). The more efficient functional PSII reaction centres enabled the rise in PSII electon transport compared to *Amaranthus* and *Eleusine* at limiting light. On the other hand *Amaranthus* showed slightly lowered efficiency of PSII function coupled with a curtailed electron transport compared to other two plants at suboptimal light. From the present study it can be safely concluded that the prime factor governing the electron transport is the functional efficiency of PSII reaction centre and its number. In *Amaranthus*, the PSI reaction centres decreased in the limiting light in mesophyll but remained constant in bundle sheath (Table 3.4). The restoration in the

number of PSI reaction centres was evident in mesophyll (Table 3.4; Figures 3.13a and 3.13b) when plants were readjusted to normal light intensity. The variation in number of PSI reaction centres (on chlorophyll basis) is a novel aspect of acclimation to lowered irradiances in *Amaranthus,* since earlier studies reported fixed PSI reaction centres during the time course of acclimation. (Leong and Anderson 1984b; De la Torre and Burkey 1990b). The observed decline in the PSI electron transport is thought to be due to reduction in PSI reaction centres in mesophyll. Factors other than lowering of the PSI reaction centres seem to control the reduction of electron transport in the bundle sheath thylakoid membranes.

The cytochrome f content denotes the estimation of cytochrome b_6/f complex. The complex decreased when the plants were acclimated to lowered irradiances and elevated when acclimated to normal growth light (Table 3.4 Figures 3.15a, 3.15b, 3.16a, 3.16b, 3.21a, 3.21b, 3.22a and 3.22b). Cytochrome b_6/f complex is a linker in establishing the efficient co-ordination between the two photosystems. The reduction of the complex probably restricts electron flow through PSI under limiting light as it was found that PSII electron flow was more sensitive than PSI in the present work. The extent of change in the stoichiometry of the supramolecular complexes varied between the three plants studied (Table 3.4). *Gomphrena* showed lesser alteration compared to other two plants. The less change in the PSII electron transport under limiting light correlated with less decline in whole chain electron transport in *Gomphrena* compared to that of other two plants (Tables 3.2 and 3.3). The less decline in levels of cytochrome b_6/f complex is consistent with lowered disturbed PSII activity (Tables 3.3 and 3.4). There was a greater decline in cytochrome b_6/f levels in bundle sheath compared to that of mesophyll (Table 3.4), which can be explained as follows : In the bundle sheath the situation appears to be a

strategy of fixed PSI reaction centre numbers and relatively disturbed PSII. This would automatically elicit a concomitant alteration in cytochrome b_6/ /. On the **other hand the** mesophyll chloroplasts have a different pattern of adjustment having deregiment in both the photosystems. Accordingly there is less alteration in the cytochrome b_6/f complex. The need for higher cyclic electron transport and ATP production in the bundle sheath is due to the C_4 mode of carbon fixation. In general more P_{700} was found in bundle sheath compared to that in the mesophyll of C_4 plants. (Mayne *et al.* 1975; Edwards *et al.* 1976). The greater requirement of ATP per *CO2* fixed was presumably due to the ATP required carbondioxide decarboxylating mechanism present in the bundle sheath. The requirement for more ATP resulted in comparatively more stable PSI complex in bundle sheath in contrast to that of mesophyll. The C_4 metabolism was responsible for greater susceptibility of PSII in contrast to PSI (Tables 3.2 and 3.3).

The changes in the stoichiometries of the supra molecular complexes was reported earlier in limiting light for other plants. (Ruhle and Wild 1979; Leong and Anderson 1984b; Wild *et al.* 1986; Chow and Hope 1987; Evans 1987b; De la Torre and Burkey 1990; Chow *et al.* 1991). However the stoichiometries of the supra molecular complexes remained fixed in pea under limiting light (Lee and Whitmarsh 1989). The present study appears to be in sharp contrast to the findings of Lee and Whitmarsh (1989) and is some what similar to other studies by Leong and Anderson (1984) and Chow and Hope (1987). The PSII/PSI ratio increased during the acclimation of plants to lowered irradiances (Melis and Harvey 1981; Anderson 1986; Mckiernan and Baker 1991; Watanabe *et al.* 1993). This was considered to be due to the enrichment of far red light in shade (Mckiernan and Baker 1991). A constant PSII/PSI ratio was observed during the acclimation of *Tradescantia* plants to lowered irradiances (Chow *et al.* 1991). On the contrary PSI/PSII ratio

increased during the acclimation of *Dunaliella* cultures to reduced irradiances, which was proposed to be due to the ATP required bicarbonate uptake. (Naus and Melis 1991). In the case of CAM/C_4 intermediate *Peperomia camptotricha*, PSI/PSII ratio increased in spongy parenchyma where energy driven C_4 pump operates. (Nishio and Ting 1993). The increase in PSI/PSII ratio was proposed to be governed more by necessity of ATP for the C_4 metabolism rather than intensity of light. From our work with C_4 plants it is proposed that an increase in PSI/PSII ratio in limiting light might be a C_4 characteristic. This study emphasizes the plasticity of composition and function of thylakoid membranes in mature plants in response to suboptimal light conditions. It refutes the static model of thylakoid membranes proposed by Lee and Whitmarsh (1989). The duration for the adjustment of thylakoid membrane structure and function varied between each of the three species studied. *Amaranthus* acclimated faster compared to *Eleusine* and *Gomphrena*. The time course of acclimation required to "fine tune" the process of photosynthesis seems to be species specific.

The photosynthetic capacity decreased under limiting light and was restored when the plants grown under reduced irradiance were re-acclimated to high irradiances (Figure 3.24; Tables 3.6 and 3.7). At given lowered irradiances the carbon fixation was much higher in *Gomphrena* compared to that of other two plants (Figure 3.24a and 3.24b; Tables 3.6 and 3.7) The reduction in the photosynthetic capacities correlated with stunted growth observed under lowered irradiances (Figures 2.1a, 2.2a, 2.3a, 2.24a and 2.24b Tables 3.6 and 3.7). A characteristically lowered growth of plants under reduced irradiances were observed for other plant species. (Boardman 1977; Rao and Das 1982). The optimization of growth is achieved through the process of acclimation in sub-optimal light.

The study resulted in identification of two modes of adjustments to the suboptimal light, *Gomphrena* (NADP-ME) type and *Amaranthus* (NAD-ME) type including *Eleusine*. The common factor for both types of acclimation is identified as counteraction of PSII impairment through augmentation of LHCII. The *Gomphrena* type of adjustment had comparatively more open PSII reaction centres which facilitate higher excitation energy capture. The Q_A is present in more oxidizing state to increase the excitation energy transfer from PSII to PSI. Therefore *Gomphrena* has registered less increase in the size of PSII antenna compared to other two plants. The electron transport and carbon fixation correlated with the PSII function. The mechanisms for such presumably superior acclimation are to be investigated further. On the other hand, *Amaranthus* and *Eleusine* partitioned more chlorophyll towards LHCII to offset comparatively more reduction in the loss of PSII function. The photosynthetic capacity was found to be lower than that of *Gomphrena* in suboptimal light thereby limiting growth and productivity under such environmental stress conditions. *Gomphrena* is identified as better performer than *Amaranthus* and *Eleusine* under suboptimal light since relatively higher photosynthesis was observed under stress conditions.

The activities of key enzymes of C_4 metabolism declined under limiting light environments in each of the plant species studied here. Plants grown at suboptimal light intensities possessed lowered enzyme activities in other plant species. (Hatch *et al.* 1969; Bassi and Passera 1982; Usuda *et al.* 1985; Cheng *et al* 1989). Ribulose bisphosphate Carboxylase oxygenase along with the gene transcripts were found to be regulated during the time course of acclimation to reduced irradiances (Priovl and Reyss 1987). We report for the first time the lowering of C_4 metabolism during the process of acclimation to lowered irradiance. The enzyme activities were restored when high light intensities were provided

to low light acclimated plants. The extent of modulation in the C_4 metabolizing enzyme activities differed in response to a given change in light intensity between the species. The rate limiting step in the acclimation of plants to reduced irradiance is identified as the decarboxylating reaction involving NAD-ME in *Amaranthus* and *Eleusine* while it is NADP-ME in *Gomphrena.* Previously it was thought that the pyruvate orthophosphate dikinase activity was the rate limiting step in maize and *Flaveria* plants grown under suboptimal light (Usuda *et al.* 1985; Cheng *et al.* 1989). The mechanism involved for preferential regulation by suboptimal light is presently unknown. In each of the three plants studied here phosphoenol pyruvate Carboxylase and pyruvate orthophosphate dikinase activities were more sensitive to changes in the light intensities compared to alanine and aspartate aminotransferases (Tables 4.1, 4.2 and 4.3). NAD-Malate dehydrogenase was least affected to changes in light intensities in *Amaranthus* or *Eleusine* since it is located in mitochondria (Tables 4.1 and 4.2). The chloroplastic and cytosolic enzymes were more prone to changes in light intensities compared to mitochondrial enzymes of C_4 metabolism. The argument can be further substantiated by the observation that the enzyme NADP-malate dehydrogenase located in chloroplasts in *Gomphrena* was more sensitive to reduced irradiances (Table 4.3). We propose differential regulation of cell organelles by the intensity of light, in C_4 plants. The regulation of levels and activities of Rubisco, the only carboxylating enzyme was an essential feature of acclimation in C_3 plants (Chow *et al.* 1991). In the present work with C_4 plants the key enzyme involved in decarboxylation of C_4 acids for the release of CO_2 to Rubisco was found to be the prime target for regulation by light. Therefore the modulation of NAD-ME and NADP-ME in the respective plants facilitates the regulation of carbon metabolism in response to available light. This regulation of C_4 enzymes by light was proposed to occur at the site

of transcription. (Nelson *et al.* 1984; Sheen and Bogorad 1987). The duration of adjustment to reduced irradiance for regulation of C_4 metabolism was however quite similar in the three plants unlike the existence of certain dissimilarities in their photochemistry.

The capacity of source was regulated through feed back inhibition of photosynthesis because of reduced sink strength due to curtailed growth in limiting light . End product of carbon assimilation plays a crucial role in biochemical feed back regulation of carbon assimilation by depriving the orthophosphate levels in chloroplasts of low light grown plants (Walker and Sivak 1986). The orthophosphate was found to be essential for regulating the electron transport and carbon metabolism (Stitt 1986; Walker and Sivak 1986; Walker and Osmond 1986; Foyer 1988; Huber 1989). The overall ATP production was lowered under reduced light conditions due to diminished PSI electron transport. Under such conditions orthophosphate recycling was limited (Walker and Sivak 1986) which brings down the activities of carbon assimilating enzymes (Figures 4.1a to 4.16b). Also it is apparent that metabolic repression of maize photosynthetic gene promoters was at the level of transcription (Sheen 1990). It may not be out of place to assume that similar mechanism may exist in the present C_4 plants for the lowered regulation of metabolism. When normal light conditions were provided the electron transport and phosphorylation were restored. The capacity of sink increased with concomitant increase in the translocation of carbon compounds on re-acclimation to normal light levels. Efficient recycling of phosphate elevated the enzyme activities to increase the source capacity at high light conditions. The changes in the enzyme activities apparently precede those changes associated with the modulation of thylakoid membrane function and composition (Figures 2.4a to 3.24b; 4.1a to 4.16b). The enzymes are activated to maximum limit by 5 days of retransfer of low irradiance grown plants to high irradiances while

the thylakoids reorganize fully only during 6-10 days. The limited phosphate recycling under these conditions brings the co-ordination between the C assimilation and primary process of photosynthesis.

The polypeptides comprising LHCII particularly 25kDa and 27kDa have increased in *Amaranthus* under limiting light and the polypeptides associated with ATP synthetase and PSII reaction centre decreased (Figures 5.1 and 5.2). When normal light was restored the light harvesting complex polypeptides decreased and polypeptides comprising ATP synthetase (55kDa and 59kDa) increased as expected along with the rise in ($D_1 D_2$) PSII reaction centre core polypeptides (Figures 5.2a, 5.2b, 5.2c and 5.2d). The decrease in density of PSII reaction centres observed for *Amaranthus* at limiting light is associated with a decrease in the assemblage of proteins comprising the PSII reaction centre. The reduction in the PSII centres in *Amaranthus* is due to decline in core protein assemblage, which is presumably an adjustment to limiting light. The finding is believed to be a novel observation.

The regulation of genes coding for the polypeptides of thylakoids by light is well established (Rodermel and Bogorad 1985) The transcript levels of psbA gene coding for the D_1 polypeptide increased on illumination. (Link 1982; Herrmann *et al* 1985; Rodermel and Bogorad 1985; Klein and Mullet 1986; Mullet and Klein 1987; Staub and Maliga 1993). In the present work the levels of mRNA coding for the D_1 polypeptides decreased when high irradiance grown plants were acclimated to lowered irradiance in *Amaranthus* (Figure 5.5). The transcript levels of psbA gene were restored when plants were re-acclimated to normal irradiances (Figure 5.5). The duration for the adjustment of the levels of D_1 polypeptide coincided with the transcript levels of the psbA gene

(Figures 5.2 and 5.5). The transcription of psbA mRNA was enhanced by **light** in **many** dicotyledonous plants (Link 1982; Thompson *et al,* 1983; Herrmann ***et*** *al.*1985; Deng and Gruissem 1987) and monocotyledonous plants (Poulsen 1983; Rodermel and Bogorad 1985; Mullet and Klein 1987; Baumgartner *et al.* 1989; Klein and Mullet **1990). The** D_1 protein synthesis was shown to be independent of light in mung bean (Thompson *et al.* 1983). The accumulation of the D_1 protein was regulated at transcriptional level (Rodermel and Bogorad 1985) and also at translational elongation (Mullet *et al.* 1990). The regulation of D_1 was also demonstrated to occur at post translational **level since** *D*\ stability requires other components of PSII core (Rochaix and Erickson 1988). The control of translation initiation was the primary mechanism for regulating the *D*\ protein accumulation in tobacco (Staub and Maliga 1993). This finding establishes that the reaction centre polypeptide synthesis in *Amaranthus* is regulated at the transcriptional level under light intensity changes (Figure 5.5). It is also consistent with the reduction in PSII reaction centres observed in the acclimation of *Amaranthus* to suboptimal light conditions.

CONCLUSIONS

1. The ability for photosynthetic acclimation to suboptimal growth irradiance levels is believed to have been established for the first time in three fully mature diverse C_4 plants. *(Amaranthus, Eleusine* and *Gomphrena)* although such acclimation is reported for C_3 plants.

2. The process and duration for acclimation has been found to be species specific in the context of present study.

3. A generalized mode of acclimation that has emerged from this study is characterized by increased size of light harvesting antenna of PSII to optimize the photosynthetic performance to the available light energy.

4. The study has resulted in identification of apparently two modes of adjustment to low light stress. These are 1) *Gomphrena* type (it is limited structural modulation combined with relatively higher performance). 2) *Amaranthus* type (greater structural modulation for matching performance.

5. Preferential enhancement of light harvesting peptides in PSII reaction centre complex was evident through repression of *D* protein transcription.

6. It is believed that fully mature leaves are able to regulate structural composition of thylakoid complexes caused by light limiting conditions. Therefore, the thylakoid membrane composition is regarded as dynamic and flexible in responsible to amelioration of environmental stresses such as light limiting conditions.

7. Present study has lead to the identification of C_4 decarboxylating enzymes NAD-ME and NADP-ME as rate limiting steps in carbon metabolism which is attuned to the stressful carbon fixation rates.

REFERENCES

Adams III, W.W., Demmig, B., Winter, K. and Schreiber, U. (1990). The ratio of variable to maximum chlorophyll fluorescence from PSII, measured at ambient temperature and at 77°K, as an indicator of the photon yield of photosynthesis. Planta 180: 166-174.

Amrutavalli, A., Reddy, R. and Das, V.S.R. (1990). Structural and functional organization of PSII particles in *Sorghum bicolor* in Proc. International Cong. Plant. Physiol. 603-609. Narosa Publishers, New Delhi.

Anderson, J.M. (1980). P_{700} content and polypeptide profile of chlorophyll protein complexes of spinach and barley thylakoids. Biochim. Biophys. Acta. 591: 113-126.

Anderson, J.M. (1986). Photoregulation of the composition, function and structure of thylakoid membranes. Ann. Rev. Plant Physiol. 37: 93-97.

Anderson, J.M. and Andersson, B. (1988). The dynamic photosynthetic membrane and regulation of solar energy conversion. Trends Biochem. Sci. 13: 351-355.

Anderson, J.M., Chow, W.S. and Goodchild, D.J. (1988). Thylakoid membrane organization in sun/shade acclimation. Aust. J. Plant Physiol. 15: 11-26.

Anderson, J.M., Goodchild, D.J. and Boardman, N.K. (1973). Composition of the photosystems and Chloroplast structure in extreme shade plants. Biochim. Biophys. Acta. 325: 573-585.

Apel, K. (1979). Phytochrome induced appearance of m-RNA activity for the apoprotein of the light-harvesting chlorophyll a/6 protein of barley *(Hordeum vulgare)*. Eur. J. Biochem. 97: 183-88.

Anion, D.I. (1949). Copper enzymes in isolated chloroplasts, Polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24: 1-15.

Barber, J., Chapman, D.J. and Telfer, A. (1987). Characterization of a PSII reaction center isolated from the chloroplasts of *Pisum sativum*. FEBS Lett. 220: 67-73.

Bassi, R. and Passera, C. (1982). Effect of growth conditions on carboxylating enzymes of *Zea mays* plants. Photosynth. Res. 3: 53-58.

Baumgartner, B.J., Rapp, J.C. and Mullet, J.E. (1989). Plastid transcription activity and DNA copy number increase early in barley Chloroplast development. Plant. Physiol. 89: 1011-1018.

Bendall, D.S., Davenport, H.E. and Hill, R. (1971). Cytochrome components in chloroplasts of higher plants. Methods Enzymol. 23: 327-344.

Bennett, J. (1983). Regulation of photosynthesis by reversible phosphorylation of the light harvesting chlorophyll a/b protein. Biochem. J. 212: 1-13.

Berry, J.O., Nikolau, B.J., Carr, J.P. and Klessing, O.F. (1986). Translational regulation of light-induced ribulose 1-5 bisphosphate Carboxylase gene expression in Amaranth. Mol. Cell. Biol. 6: 2347-2335.

Berzborn, R.J., Muller, D., Roos, P. and Andersson, B. (1981). Significance of different quantitative determinations of photosynthetic ATP synthetase CF_1 for heterogenous, CF_1 distribution and grana formation. In photosynthetic membranes eds. G. Akoyunoglou 3: 107-120. Philadelphia: Balban Inst. Sci. Serv. 1042.

Bhaskar, C.V.S. and Das, V.S.R. (1987). Low light stress induced rate limiting steps in the thylakoids of grain sorghum. Photosynthetica. 21: 466-571.

Björkman, 0. (1973). Comparitive studies on photosynthesis in higher plants. In **pho**tophysiology ed. A.C. Glese, 8: 1-63, New York: Academic.

Björkman, 0. (1981). Response to different quantum flux densities. In encyclopedia of plant physiology: Physiological Plant Ecology (NS) ed. O.L. Lange, P.S. Nobel, C.B. Osmond, H. Ziegler, 12A: 57-107. Berlin/Heidelberg: Springer-Verlag.

Björkman, 0. (1987). High irradiance stress in higher plants and interaction **with other** stress factors. In: Progress in Photosynthesis research. Ed. J. Biggins, IV: 12-18, Martinus Nijhoff, Dordrect.

Björkman, 0., Boardman, N.K., Anderson, J.M., Thome, S.W., Goodchild, D.J. and Pyliotis, N.A. (1972). Effect of light intensity during growth of *A triplex patula* on the capacity of photosynthetic reactions, Chloroplast components and structure. Carnegic inst. Yearb. 71: 115-135.

Björkman, 0. and Badger, M. (1978). Carnegie Inst. Washington year book. WO 78: 145.

Björkman, 0. and Demmig, B. (1987). Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77°K among vascular plants of diverse origins. Planta. 170: 489-504.

Boardman, N.K. (1977). Comparitive photosynthesis of sun and shade plants. **Ann.** Rev. Plant Physiol. 28: 355-377.

Boardman, N.K., Anderson, J.M. and Goodchild, D.J. (1978). Chlorophyll protein complexes and structure of mature **and** developing chloroplasts. **Curr. Top Bioenerg. 8:** 35-109.

Boardman, N.K., Björkrnan 0., Anderson J.M., Goodchild, DJ. and Thome, S.W. (1974). Photosynthetic adaptation of higher plants to light intensity: Relationship between Chloroplast structure, composition of the photosystems and photosynthetic rates. In Proc. 3rd Intl. Congress Photosynthesis. ed M. Avron. 1809-1827. Amsterdam : Elsevier.

Boyer, J.S. (1982). Plant productivity and environment. Science. 218: 443-448.

Braumann, T.H., Weber, G. and Grimme, L.H. (1982) Carotenoid and chlorophyll composition of light harvesting and reaction centre proteins of the thylakoid membrane. Sigmoidicity, energy transfer and the slow phase. Photobiochem. Photobiophys. 4: 1-8.

Burkey, K.O. and Wells, R. (1991). Response of soy bean photosynthesis and Chloroplast membrane function to canopy development and mutual shading. Plant Physiol. 97: 245-252.

Butler, W.L. and Kitajima, M. (1975). Energy transfer between photosystem II and I in chloroplasts. Biochim. Biophys. Acta. 396: 72-85.

Butler, P.J.G. and Kuhlbrandt, W. (1987). Determination of the aggregate size in detergent solution of the light harvesting chlorophyll *a/b* protein complex from Chloroplast membranes. Proc. Natl. Acad. Sci. (USA) 85: 3797-3801.

Caemmerer, S.V. and Farquahar, G.D. (1984). Effects of partial defoliation changes of irradiance during growth, short term water stress and growth at enhanced p (Co_2) on the photosynthetic capacity of the leaves of *Phaseolus vulgaris.* L. Planta. 160: 320-329.

Chabot, B.F., Jurik, T.W. and Chabot, J.F. (1979), Influence of instantaneous and integrated light flux density on leaf anatomy and photosynthesis. Amer. J. Bot. 66: 940-945.

Cheng, S.H., Moore, B.d., Wu, J., Edwards, G.E. and Ku, M.S.B. (1989). Photosynthetic plasticity in *Flaveria brownii*. Growth irradiance and expression of C_4 photosynthesis. Plant Physiol. 89: 1129-1135.

Chow, W.S., Adamson H.Y. and Anderson, J.M. (1991). Photosynthetic acclimation of *Tradescantia albiflora* to growth irradiance : Lack of adjustment of light-harvesting components and its consequences. Physiol. Plant. 81: 175-182.

Chow, W.S. and Anderson, J. M. (1987a). Photosynthetic responses of *Pisum sativum* to an increase during growth. I. Photosynthetic activities. Aust. J. Plant. Physiol. 14: 1-8.

Chow, W.S. and Anderson, J.M. (1987b). Photosynthetic response of *Pisum sativum* to an increase in irradiance during growth. II. Thylakoid membrane components. Aust. J. Plant Physiol. 14: 9-19.

Chow, W.S. and Hope, A.B. (1987). The stoichiometries of supra molecular complexes in thylakoid membranes from spinach chloroplasts. Aust. J. Plant Physiol. 14: 21-28.

Davies, E.C., Chow, W.S., Lefay, J.M. and Jordan, B.R. (1986). Acclimation of tomato leaves to change in light intensity; effect on the function of thylakoid membrane. J.Exp. Bot. 37: 211-220.

Davies, E.C., Jordan, B.R., Partis, M.D. and Chow, W.S. (1987). Immunochemical investigation of thylakoid coupling factor protein during photosynthetic acclimation to irradiance. J. Exp. Bot. 38: 1517-1527.

Das, V.S.R. and Reddy, A.R. (1988). Photosynthetic productivity in field crops. Plant Physiol. Biochem. 15(1): 1-17.

De la Torre, W. R. and Burkey, K. 0. (1990a). Acclimation of barley to changes in light intensity : Chlorophyll organization. 24: 117-125.

De la Torre, W.R. and Burkey, K.O. (1990b). Acclimation of barley to changes in light intensity. Photosynthetic electron transport activity and components. Photosynth. Res. 24: 127-136.

Deng, X. W. and Gruissem, W. (1987). Control of plastid gene expression during development; The limited role of transcriptional regulation. Cell. 49: 379-387.

Edwards, G.E. and Guiterrez, M. (1973). Metabolic activities in extracts of mesophyll and bundle sheath cells of *Panicum maliaceum* in relation to C_4 dicarboxylic pathway of photosynthesis. Plant Physiol 50: 728-732.

Edwards, G.E., Huber, S.S., Kuss, B. Ratnam, C.K.M., Guiterrez, M. and Mayne, B.C. (1976). Variation in photochemical activities of C_4 plants in relation to CO_2 fixation. In: CO_2 metabolism and plant productivity. eds. Burris, R.N. and Black, C.C. 83-112. University Park Press, Baltimore.

Eskins, K., Duysen, M., Dybas, L. and Marcarthy, S. (1985). Light quality effects on corn Chloroplast development. Plant Physiol 77: 29-34.

Evans, J.R. (1987a). The dependence of quantum yield on wavelength and growth irradiance. Aust. J. Plant Physiol. 14: 69-79.

Evans, J.R. (1987b). The relationship between electron transport component and photosynthetic capacity in pea leaves grown at different irradiances. Aust. J. Plant Physiol. 14: 157-170.

Evans, J.R. (1993a). Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy I. Canopy characteristics. Aust. J. Plant Physiol. 20: 55-67.

Evans, J.R. (1993b). Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy II. Stability through time and comparision with a theoretical optimum. Aust. J. Plant Physiol. 20: 69-82.

Falkowski, P.G. and Laroche, J. (1991). Acclimation to spectral irradiance. J. Phycol. 27: 8-14.

Feinberg, A.P. and Vogelstein, B. (1983). A technique for radiolabelling UNA restriction endonuclease fragments to high specific activity. Anal Biochem. 132: 6-13.

Feinberg, A.P. and Vogelstein, B. (1984). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem. 137: 266-267

Ferguson, D.L. and Burke, J.J. (1991). Influence of water and temperature stress on the temperature dependence of the reappearance of variable fluorescene following illumination. Plant Physiol. 97: 188-192.

Ferrar, P.J. and Osmond, C.B. (1986). Nitrogen supply as a factor influencing photoinhibition and photosynthetic acclimation after transfer of shade grown *Solanum dulcamara* to bright light. Planta. 168: 563-570.

Fotinou, C. and Ghanotakis, D. (1990). A preparative method for the isolation of 43kDa, 47kDa and $D_1 - D_2$ — *cyt* b_{559} species directly from thylakoid membranes. Photosynth. Res. 25: 141-145.

Foyer, C.H. (1988). Feed back inhibition of photosynthesis through source sink regulation in leaves. Plant Physiol. Biochem. 26: 483-492.

Freeman, J., Marquez, A.J., Wallsgrouo, M., Saarelainen, R. and Forde, B.G. (1990). Molecular analysis of barley mutants deficient in Chloroplast glutamine synthetase. Plant. Mol. Biol. 14: 297-311.

Furbank, R.T. and Foyer, CH. (1988). C_4 plants as valuable model experimental systems for the study of photosynthesis. New Phytol. 109: 265-277.

Genty, B., Briantais, J.M. and Baker, N.R. (1989). The relationship between the quantum yield of photosynthetic electron transport and the quenching of chlorophyll fluorescence. Biochim. Biophys. Acta. 990: 87-92.

Ghirardi, M. L., Mahajan, S., Sapori, S. K., Edelman, M. and Mattoo, A. K. (1993). Photosystem II reaction centre particle from *Spirodela* stroma lamellae. J. Biol. Chem. 268: 5357-5360.

Gilmartin, P.M., Sarakin, L., Memelink, J. and Chua, N.H. (1990). Molecular light switches for plant genes. Plant Cell. 2: 369-378.

Gounaris, K., Brain, A.P.R., Quinn, P. J. and Williams, W. P. (1984). Structural reorganization of Chloroplast thylakoid membranes in response to heat stress. Biochim. Biophys. Acta. 766: 198-208.

Golbeck, J.H. (1992). Structure and function of photosystem I. Ann. Rev. Plant. Physiol. Plant Mol. Biol. 43: 293-324.

Goodchild, D.J., Andersson, B. and Anderson, J.M. (1985a). Immunocytochemical localization of polypeptides associated with oxygen evolving system of photosynthesis. Eur. J. Cell Biol. 36: 294-298.

Goodchild, D. J. Andersson, B. and Anderson, J. M. (1985b). Immunocytochemical localization of the cytochrome b_6/f complex of Chloroplast thylakoid membranes. Cell Biol. Int. Rep. 9: 715-721.

Grahl, H. and Wild, A. (1975) Studies on the content of P_{700} and cytochromes in *Sinapis alba* during growth under two different light intensities. In: Environmental and biological control of photosynthesis. ed. R.R. Marcelle, 107-113.

Green, B.A., Allred, D.R., Morishinge, D.T. and Staehelin, L.A. (1988). Hierarchial response of light harvesting chlorophyll proteins in a light sensitive clilorophyll b - deficient mutant of maize. Plant Physiol. 87: 357-364.

Greer, D.H. and Laining, W.A. (1989). Photoinhibition of photosynthesis in intact kiwi fruit *(Actinidia deliciosa)* leaves : Effect of growth temperature on photoinhibition and recovery. Planta. 180: 32-39.

Gutierrez, M., Gracen, V.E. and Edwards, G.E. (1974). Biochemical cytological relationships in C_4 plants. Planta. 119: 279-300.

Hatch, M.D., Kagawa, T. and Craig, S. (1975). Sub division of C_4 pathway species based on differing C_4 acid decarboxylating systems and ultra structural features. Aust. J. Plant Physiol. 2: 111-128.

Hatch, M.D., Slack, C.R. and Bull, T.A. (1969). Light induced changes in the content of some enzymes of the C_4 - dicarboxylic acid pathway of photosynthesis and its effect on other characteristics of photosynthesis. Phytochem. 8: 697-706.

Hatch, M.D., Suzuki, G.E. and Edwards, G. E. (1982). Determination of NAD-ME in leaves of C_4 plants. Effect of malate dehydrogenase and other factors. Plant Physiol. 81: 899-895.

Herrmann, R.G., Westhoff, P., Alt. J., Tittgen. J. and Nelson. N. **(1985). Thylakoid** membrane proteins and their genes. In: Molecular form and function of the plant genome. eds. Lvan Vloten Doting, G.S.P. Groot, T.C. Hall, 233-256, Plenum Press, New York.

Hiyama, T. and Ke, B. (1972). Difference spectra and extinction coefficients of P_{700}. Biochim. Biophys. Acta. 267: 160-171.

Hodges, M. and Barber, J. (1983). Photosynthetic adaptation of pea plants grown at different light intensities : state 1 and state 2 transitions and associated chlorophyll fluorescence changes. Planta. 157: 166-173.

Huber, S. (1989). Biochemical mechanisms for regulation of sucrose accumulation in leaves during photosynthesis. Plant Physiol. 91: 656-662.

Iglesias, A.A. and Andrea, C.S. (1989). Purification of NADP-ME and PEP Carboxylase from sugar cane leaves. Plant Cell Physiol. 30: 399-405.

Ikeuchi, M., Yuasa, M. and Inoue, Y. (1985). Simple and discrete isolation of an O_2 evolving PSII reaction centre complex retaining Mn and extrinsic 33 kDa protein. FEBS Letts. 185: 316 - 322.

Jenkins, C.I., Gallagher T.F., Hartley M.R., Bennett, J. and Ellis, R.J. (1984). Photo regulation of gene expression during Chloroplast biogenesis. In: Advances in photosynthesis Research ed. C. Sysbesma, 4: 863-872. The Hague. Nijhoff/Dr. W. Junk.

Kanai, R. and Edwards, G.E. (1973). Separation of maize leaves mesophyll protoplasts and bundle sheath cells. Plant Physiol. 51: 1133-1137.

Klein, R.R. and Mullet, J.E. (1986). Regulation of Chloroplast encoded chlorophyll binding protein translation during higher plant Chloroplast biogenesis. J. Biol. Chem. 261: 11138-11145.

Klein, R.R. and Mullet, J.E. (1990). Light-induced transcription of Chloroplast genes. psbA transcription is differentially enhanced in illuminated barley. J. Biol. Chem. 265: 1895-1902.

Krause, G.H., Somesalo S., Zumbusch E., Weyers, B. and Laasch, H. (1990). On the mechanism of photoinhibition in chloroplasts. Relationship between changes in fluorescence and activity of PSII. J. Plant Physiol. 136: 472-479.

Kuhlemeier, C. (1992). Transcriptional and post-transcriptional regulation of gene expression in plants. Plant. Mol. Biol. 19: 1-15.

Kuhlemeier, C, Green, P.J. and Chua, N.H. (1987). Regulation of gene expression in higher plants. Ann Rev. Plant Physiol. 38: 119-221.

Kuwabara, T. and Murata, N. (1982). Inactivation of photosynthetic oxygen evolution and concomitant release of three polypeptides in the photosystem II particles of spinach chloroplasts. Plant Cell. Physiol. 23: 533-539.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of bacteriophage T_4. Nature 227: 680-685.

Lam, E., Oritz, W., Mayfield, S. and Malkin, R. (1984). Isolation and characterization of a light-harvesting chlorophyll *a/b* protein complex associated with photosystem I. Plant Physiol. 74: 650 - 655.

Langdale, J.A., Zelitch, I., Miller, E . and Nelson, T. (1988). Cell **position and light** influences C_4 versus C_3 patterns of photosynthetic gene expression in maize. **EMBO** J. 7: 3643-3651.

Larsson, U.K., Anderson, J.M. and Andersson, B. (1987) Variations in the relative content of the peripheral and inner light-harvesting chlorophyll *a/b* protein complex (LHC II) subpopulations during thylakoid light adaptation **and** development. Biochim. Biophys. Acta. 894: 69-74.

Lee, W. and Whitmarsh, J. (1989). Photosynthetic apparatus of pea thylakoid membranes. Response to growth irradiance. Plant Physiol. 89: 932-940.

Leong, T.Y. and Anderson, J.M. (1983). Changes in composition and function of thylakoid membranes as a result of photosynthetic adaptation of chloroplasts from pea plants grown under different light conditions. Biochim. Biophys. Acta. 723: 391-399.

Leong, T.Y. and Anderson, J.M. (1984a). Adaptation of the thylakoid membranes of **pea** chloroplasts to light intensities I. Study on the distribution of chlorophyll-protein complexes. Photosynth. Res. 5: 105-115.

Leong, T.Y. and Anderson, J.M. (1984b). Adaptation of the thylakoid membrane of pea chloroplasts to light intensities II. Regulation of electron transport capacity, electron carriers, coupling factor (CF_1) activity and rates of photosynthesis. Photosynth. Res. 5: 117-128.

Levy, I. and Gnatt, E. (1988). Light acclimation in *Porphyridium purpureum* (Rhodophyta) growth, photosynthesis and phycobilisomes. J. Phycol. 24: 488-495.

Lichtenthaler, H.K. (1981). Adaptation of leaves and chloroplasts to high quanta fluence rates. In: Photosynthesis ed. G. Akoyunoglou, 6: 278-88. Philadelphia Balaban Inst. Sci. Ser.

Lichtenthaler, H.K., Kuhn, G., Prenzel, U., Burchmann, C. and Meier, D. (1982). Adaptation of Chloroplast ultrastructure and of chlorophyll-protein levels to high light and low light growth conditions. Z. Naturforsch. Tul C. 37: 464-475.

Lichtenthaler, H.K. and Meier, D. (1984). Regulation of Chloroplast photomorphogenesis by light intensity and light quality. In: Chloroplast biogenesis, ed. R.J. Ellis, pp. 261-281, Cambridge: Cambridge Univ.

Link, G. (1982). Phytochrome control of plastid mRNA in mustard *Sinapis alba* L. Planta. 154: 81-86.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Malkin, R., Ortiz, W., Lam, R. and Bonnerjea, J. (1985). Structural organization of thylakoid membrane electron transfer complexes: Photosystem I. Physiol. Veg. 23: 619-625.

Marder, J.B., Chapman, P.J., Telfer, A., Nixon, P.J. and Barber, J. (1987). Identification of psbA and psbD gene products, $D\backslash$ and D_2 as reaction centre proteins of photosystem II. Plant. Mol. Biol. 9: 325-333.

Mattoo, A.K., Marder, J.B., Gaba, V. and Edelman, M. (1986). Control of 32 kDa thylakoid protein degradation as a consequence of herbicide binding to its receptor. In: regulation of Chloroplast differentiation, ed. G. Akoyunoglou, 607-613. New York, Alan, R. Liss. Inc.

Mayne, B.C., Dee, A.M. and Edwards, G.E. (1975). Photosynthesis **in** mesophyll protoplasts and bundle sheath cells of various type (SIC) of C_4 plants. Fluorescence emission spectra, delayed light emission and P_{700} content. Z. Pflanzenphysiol. 74; 275-291.

McDonnel, A. and Staehelin, L.A. (1980). Adhesion between liposomes mediated by the chlorophyll *a/b* light harvesting complex isolated from the Chloroplast membranes. J. Cell Biol. 84: 40-56.

Mckiernan, M. and Baker, W.R. (1991). Adaptation to shade of the light-harvesting apparatus in *Silene dioca*. Plant Cell Environ. 14: 205-212.

Melis, A. and Harvey, G.W. (1981). Regulation of photosystem stoichiometry, chlorophyll-a and chlorophyll-b content in relation to Chloroplast ultrastructure. Biochim. Biophys. Acta. 637: 138-145.

Meyer, R.F. and Boyer, J.S. (1972). Sensitivity of cell division and cell elongation to low water potentials in soybean hypocotyls. Planta. 108: 77-87.

Miller, A.F. and Brudvig, G.W. (1991). A guide to electron paramagnetic resonance spectroscopy of photosystem II membranes. Biochim. Biophys. Acta. 1056: 1-18.

Morgan, J.M. (1977). Differences in osmoregulation between wheat genotypes. Nature (London), 270: 234-235.

Mullet, J., Burke, J.J. and Arntzen, C (1980). Chlorophyll proteins of Photosystem I. Plant. Physiol. 65: 814-822.

Mullet, J.E., Gamble, K.P. and Klein, R.R. (1990). Chlorophyll regulates accumulation of the plastid encoded chlorophyll apoproteins. CP43 and D_1 by **increasing** apoprotein stability. Proc. Natl. Acad. Sci., (USA) 7: 4038-4042.

Mullet, J.E. and Klein, R.R. (1987). Transcription and RNA stability are important determinants of higher plant Chloroplast RNA levels. EMBO J. 6: 1571-1579.

Nanba, 0. and Satoh, K. (1987). Isolation of Photosystem II reaction centre consisting of D_1 and D_2 polypeptides and Cytochrome b_{559}. Proc. Natl. Acad. Sci. (USA) 84:109-112.

Naus, J. and Melis, A. (1991). Changes of photosystem stoichiometry during cell growth in *Dunaliella salina* cultures. Plant Cell Physiol. 32: 569-575.

Nechushtai, R., Schuster, G., Nelson, N. and Ohad, I. (1986). Photosystem I reaction centers from maize bundle sheath and mesophyll chloroplasts lack subunit III. Eur. J. Biochem. 159: 157-161.

Nelson, T., Harpster, M., Mayfield, S.and Taylor, W.C. (1984). Light regulated gene expression during maize leaf development. J. Cell Biol. 98: 558-564.

Nisho, J.N. and Ting, I.P. (1993). Photosynthetic characteristics of the palisade mesophyll and spongy mesophyll in the CAM/C_4 intermediate plant *Peperomia camptotricha*. Bot. Acta. 106: 120-125.

Ogren, E. and Öquist, G. (1984). Photoinhibition in *Lemna gibba* as induced by the interaction between light and temperature III. Chlorophyll fluorescence at 77°K. Physiol. Plant. 62: 193-200.

Ollander, C, Hundal, T., Andersson, B., Huner, N.P.A. and Öquist, G. (1993). PSII reaction centre stays intact during low temperature photoinhibition. Photosynth. Res. 35: 191-200.

Öquist, G., Greer, D.H. and Ogren, E. (1987). Light stress at low temperature. In: Photoinhibition, eds. Kyle, D.J., Osmond, C.B. and Arntzen, C.J.,. 67-88, Elsevier, Amsterdam.

Peter, G.F. and Thornber, J.P. (1991). Biochemical composition and organization of higher plant photosystem II light harvesting pigment-proteins. J. Biol. Chem. 266: 16745-16754.

Poulsen, C. (1983). The barley Chloroplast genome: Physical structure **and** transcriptional activity *in vivo.* Carlsberg Res. Commun. 48: 57-80.

Powles, S.B. (1984). Photoinhibition of photosynthesis induced by visible light. Ann. Rev. Plant Physiol. 35: 15-44.

Powles, S.B. and Björkman, 0. (1982). Photoinhibition of photosynthesis: effect on chlorophyll fluorescence at 77°K in intact leaves and in Chloroplast membranes of *Nerium oleander,* Planta. 156: 97-107.

Prioul, J.L. and Reyss, A. (1987). Acclimation of ribulose bisphosphate Carboxylase and mRNAs to changing irradiance in adult tobacco leaves. Plant Physiol. 84: 1238-1243.

Raghavendra, A.S. and Das, V.S.R. (1976). Diversity in the biochemical and biophysical characteristics of C_4 dicotyledonous plants. Ind. J. Plant Physiol. 19: 101-112.

Raghavendra, A. S. and Das, V.S.R. (1993). C_4 photosynthesis and C_3 - C_4 intermediacy: Adaptive strategies for semi arid tropics. In: Photosynthesis photoreactions to plant productivity, ed. Abrol, Y. P., Mohanty, P. and Govindjee, 317-338. Oxford and IBH publishing house.

Ramus, J. (1981). The capture and transmission of light energy 、 In: The biology of sea weeds, eds. C.S. Lobban and M.J. Wynne. 458-492. Blackwell Scientific Publications, Oxford.

Rao, A.N. and Das, V.S.R. (1982). Chlorophyll content and photochemical activities of three *Sorghum* cultivars grown under three irradiances. Photosynthetica. 16: 145-147.

Richard, G.E. and Hall, D.O. (1987). Photoinhibition at chilling temperature, in intact leaves and isolated chloroplasts of barley. In: Progress in photosynthesis reasearch, ed. J. Biggns, IV, 39-42, Martinus Nijhoff, Dordrecht.

Rochaix, J.D. and Erickson, J. (1988). Function and assembly of photosystem II: genetic and molecular analysis. Trends Biochem. Sci. 13: 56-59.

Rodermel, S.R. and Bogorad, L. (1985). Maize plastid photogenes: Mapping and photoregulation of transcription levels during light induced development. J. Cell. Biol. 100: 463-476.

Ruhle, W. and Wild, A. (1979). Measurement of cytochrome / and P_{700} in intact leaves of *Sinapis alba* grown under high light and low light conditions. Planta 146: 377-385.

Sambrook, J., Fritsch, E.F., Maniatis, R. (1989). Molecular cloning: A laboratory manual, 2nd ed. cold Spring Harbor, Laboratory press. Cold Spring Harbor, New York.

Schaffner, A. and Sheen, J. (1991). Maize rbcS promoter activity depends on sequence elements not found in dicot rbcS promoters. Plant Cell. 3: 997-1012.

Schantz, R. and Bogorad, L. (1988). Maize Chloroplast genes ndhD, ndhE and psaC. sequences, transcripts and transcript pools. Plant Mol. Biol. 11: 239-247.

Scheller, V.H. and Moller, B.L. (1990). Photosystem I polypeptides. Physiol. Plant. 78: 484-494.

Sharma, P.K. and Hall, D.O. (1992). Effect of high-irradiance stress on primary photochemistry and light regulated enzymes of photosynthetic carbon metabolism. J. Plant. Physiol. 139: 719-726.

Sheen, J. (1990). Metabolic repression of transcription in higher plants. The Plant Cell. 2: 1027-1038.

Sheen, J.Y. and Bogorad, L. (1987). Differentia] expression of C_4 pathway genes in mesophyll and bundle sheath cells of greening maize leaves. J. Biol. Chem. 262: 11726-11730.

Shuvalov, V.A., Heber, U. and Schreiber, U. (1989). Low temperature photochemistry and spectral properties of PSII reaction center complex containing the proteins D_1 and D_2 and two hemes of *Cyt* b_{559}. FEBS Lett. 258: 27-31.

Staehelin, L.A. (1986). Chloroplast structure and supramolecular organization of photosynthetic membranes. In: Encyclopedia of plant physiology, eds. L.A. Staehelin, C.J. Arntzen. 19: 1-84, Springer-Verlag, Berlin.

Staub, J. M. and Maliga, P. (1993). Accumulation of D_1 polypeptide in tobacco plastids is regulated via the untranslated region of the psbA mRNA. EMBO J. 12: 601-606.

Stitt, M. (1986). Regulation of photosynthetic sucrose synthesis: Integration, adaptation and limits. In: Phloem transport, eds. J. Cronshaw, W.J. Lucas, R.J. Lucas and R.T. Gianquintz, 331-347, New York. Alan, R. Liss, Inc.

Terashima, I. and Inoue, Y. (1984). Comparitive photosynthetic properties of palisade tissue chloroplasts and spongy tissue chloroplasts of *Camellia japonicah.* Functional adjustment of the photosynthetic apparatus to light environment within a leaf. Plant Cell Physiol. 25: 555-563.

Thayer, S.S. and Björkmann, 0. (1990). Leaf xanthophyll content and composition in sun and shade determined by HPLC. Photosynth. Res. 23: 331-343.

Thompson, W. F., Everett, M., Polans, N. O., Jorgensen, R. A. and Palmer, J. (1983). Phytochrome control of RNA levels in developing pea and mung bean leaves. Planta. 158: 487-500.

Thompson, W.F. and White, M. J. (1991). Physiological and molecular studies of light regulated nuclear genes in higher plants. Ann. Rev. Plant Physiol. Mol. Biol. 42: 423-466.

Tobin, E.M. (1981). Phytochrome mediated regulation of messenger RNAs for the small subunit of ribulose 1,5 bisphosphate Carboxylase and the light harvesting chlorophyll *a/b* protein in *Lemna gibba.* Plant. Mol. Biol. 1: 35-51.

Tobin, E.M. and Silverthorne, J. (1985). Light regulation of gene expression in higher Plants. Ann. Rev. Plant. Physiol. 36: 569-593.

Usuda, H., Ku, M.S.B. and Edwards, G.E. (1984). Activation of NADP-MDH pyruvate pi dikinase and fructose 1,6 bis phosphatase in relation to photosynthetic rate in maize. Plant Physiol. 76: 238-243.

Usuda, H. Ku, K.S.B. and Edwards, G.E. (1985). Influence of light intensity during growth on photosynthesis and activity of several key photosynthetic enzymes in *CA* plant *Zea mays.* L. Physiol. Plant. 63: 65-70.

Vainstein, A., Ferreira, P., Peterson, C.C., Verbeke, J.A. and Thornber, J.P. (1989). Expression of the major light-harvesting chlorophyll *a/b* protein and its import into thylakoids of mesophyll and bundle sheath chloroplasts of maize. Plant. Physiol. 89: 602-609.

Walker, D.A. and Osmond, C.B. (1986). Measurement of photosynthesis *in vivo* with a leaf disc electrode: correlations between light dependence of steady state photosynthetic O_2 evolution and chlorophyll a fluorescence transients. Proc. R. Soc. Lond. B. 227: 267-280.

Walker, D.A. and Sivak, M.N. (1986). Photosynthesis and phosphate: A cellular affair? Trends Biochem. Sci. 11: 176-179.

Watanabe, N., Masakishirota, C.F. and Furuta, Y. (1993). Changes in chlorophyll, thylakoid proteins and photosynthetic adaptation to sun and shade environments in diploid and tetraploid *Oryza punctata*, Kotschy and diploid *Oryza eichingtri* Peter. Plant Physiol. Biochem. 31: 469-474.

Wild, A. (1979). Physiology of Photosynthesis in hylies, plants. The adaptation of photosynthesis to light intensity and light quality. Berr. Dtrch. Bot. Ges. 92: 341-364.

Wild, A., Hopfner, M., Ruhle, W. and Ritchter, M. (1986). Changes in the stoichiometry of photosystem II components as an adaptive response to high-light and low-light conditions during growth. Z. Naturforsch 41C: 597-603.

Wynn, R.M., Luong, C. and Malkin, R. (1989). Maize Photosystem I. Identification of the subunit which binds plastocyanin. Plant Physiol. 91: 445-449.