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Photosynthetic characteristics of chloroplasts of primary wheat leaves grown under different irradiance

Rajendra K. BEHERA and Nakul K. CHOUDHURY*

School of Life Sciences, Sambalpur University, Jyotivihar - 768 019, Sambalpur, India

Abstract

The rate of accumulation of total chlorophyll (Chl) and carotenoids (Car) of leaves grown under high irradiance, HI (30 and 45 W m^{-2}) was faster than at moderate irradiance, MI (15 W m^{-2}). However, the senescence phase started earlier in the samples and proceeded at a faster rate. Chl *a/b* and Chl (*a+b*)/Car values showed faster loss of Chl *a* (compared to Chl *b*) and Chl (*a+b*) (compared to Car) in HI leaves. Protein accumulation and loss were also similar to that of Chl (*a+b*) content. Increase in Chl fluorescence during the development phase may suggest a gradual change in thylakoid organisation, however, the temporal kinetics were different in HI and MI samples. Increase in fluorescence polarisation during senescence of HI leaves compared to the control (MI) suggests conversion of thylakoid membranes to gel phase. Chloroplasts prepared from HI seedlings showed higher rate of photochemical activities, however, the activity declined earlier and at faster rate compared to the control.

Additional key words: carotenoids; chlorophyll fluorescence; Hill activity; leaf ageing; membrane polarisation; senescence; *Triticum aestivum*; wheat.

Introduction

Variation in radiation quality and duration or irradiance cause significant change in pattern of leaf growth and senescence. Leaves of high irradiance (HI) grown plants differ in morphology, composition, and photosynthetic characters from the low irradiance (LI) ones (*e.g.*, Lichtenthaler 1981, Lichtenthaler *et al.* 1981). HI leaves have small leaf size, high dry mass, low water content, and high Chl *a/b* ratio. Their development is much faster than that of leaves of LI plants. HI leaves possess sun-type chloroplasts that are adapted for a higher photosynthetic quantum conversion than leaves of moderate irradiance (MI) or LI plants (Meier and Lichtenthaler 1981, Lichtenthaler *et al.* 1984, Čajánek *et al.* 1999).

HI causes injury to photosynthetic apparatus. Sun plants grow better at HI than at LI (Powles 1984, Demmig-Adams and Adams 1992, Björkman and Demmig-Adams 1993). Transfer of sun plant growing in LI to HI results in an upward shift of photosynthesis to accommodate and utilise the increased supply of radiant energy.

Carotenoids (Car) are accessory pigments in photosynthesis. The other important function of Car is photoprotection of Chl against photooxidative degradation (Demmig-Adams 1990, Gilmore 1997, Anderson *et al.* 1997). In contrast to wild type organisms having normal pigment composition, mutants lacking Car suffer serious photooxidative damage, often resulting in death. This suggests photoprotective role of Car in plants under irradiance stress.

The objective of this work was to study the effect of irradiance on pigment composition and photosynthetic characters of chloroplasts of plants grown under HI.

Materials and methods

Wheat (*Triticum aestivum* L. var. Sonalika) seedlings were grown in a culture room on sterilised cotton in Petri dishes at 25±2 °C under continuous "white light" at irradiances of 15 W m^{-2} (MI), 30 W m^{-2} (HI-1), and 45 W m^{-2} (HI-2). Primary leaves were harvested at 24-h intervals for various experiments. Pigments were extracted in 80 % chilled acetone.

The amounts of Chl (*a+b*) and Car were estimated

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*Author for correspondence; fax: (0663) 430158, e-mail: rajubb7@hotmail.com
Abbreviations: Car – carotenoids; Chl – chlorophyll; DCPIP - 2,6-dichlorophenol indophenol; HI – high irradiance; LHCP - light-harvesting chlorophyll-protein; LI – low irradiance; MI – moderate irradiance; PFD - photon flux density.
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spectrophotometrically according to Lichtenthaler (1987). The sodium chloride soluble proteins of leaves were estimated by Coomassie brilliant blue G-250 method as described by Bradford (1976). Bovine serum albumin was used as standard.

About 25 leaves were homogenised in pre-chilled mortar and pestle with ice-cold isolation medium. The isolation medium contained 0.4 M sucrose, 0.01 M EDTA-Na, and 0.1 M phosphate buffer (pH 7.8). After homogenisation, the homogenate was squeezed through cheesecloth and the filtrate was centrifuged at 500×*g* for 1 min. The supernatant was again centrifuged at 1000×*g* for 10 min. The pellet containing chloroplasts was collected in a small volume of homogenising medium.

The room temperature Chl fluorescence emission of chloroplasts was measured by a spectrofluorometer (*Hitachi*, model *650-40*) following Swain *et al.* (1990). The samples were excited at 450 nm and emission was recorded at 685 and 735 nm. Chloroplasts equivalent to 10 μg Chl in 3 cm^3 of suspension medium were taken for all experiments. For measuring the fluorescence polarisation, the chloroplast suspension was excited at 620 nm and polarisation (P) was recorded at 685 nm. P was calculated using the formula of Swain *et al.* (1990) with modification.

The 2,6-dichlorophenol indophenol (DCPIP) photoreduction by isolated chloroplasts was measured spectrophotometrically as described by Swain *et al.* (1990). The 3 cm^3 of reaction mixture contained chloroplasts equivalent to 15 μg of Chl, 15 μM DCPIP,100 mM KCl, 0.1 mM $MgSO_4$, and 10 mM Na-phosphate buffer (pH 6.8). The incident radiation beam was passed through a water filter to minimise infrared radiation. The photoreduction of the dye was measured at 600 nm.

Results

In the leaves of seedlings grown in HI-2, Chl accumulated faster and also declined earlier compared to HI-1 and MI leaves (Fig. 1). The steady-state period

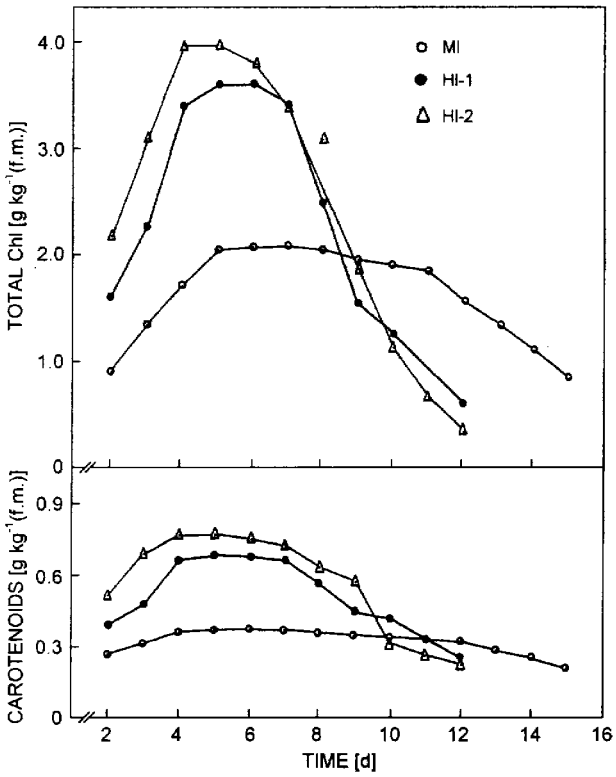

Fig. 1. Contents of chlorophyll (Chl) (*a*+*b*) and carotenoids of primary leaves of wheat seedlings grown in laboratory under different irradiances.

(maximum pigment concentration) remained longer in MI plants compared to HI-1 and HI-2 plants. However, the steady state was reached one day earlier in HI-2 plants. The rate of decline in MI (control) from 8 d was much slower compared to HI-1 and HI-2. Plants grown under HI-2 showed a higher content of Car compared to the plants grown under HI-1 and MI. Loss of Car started from 5 and 6 d in the seedlings grown under HI-2 and HI-1, respectively. Car amount declined rapidly in HI-2 samples compared to HI-1 and MI.

Table 1. The chlorophyll (Chl) *a*/*b* and Chl (*a*+*b*)/carotenoids (Car) ratios of primary wheat leaves of seedlings grown under moderate (MI) and high (HI-1 and HI-2) irradiances. Exceptionally, mature leaves of MI plants were analysed on the 7th d.

	Stage	MI	HI-1	HI-2
Chl *a*/*b*	Young (2 d)	2.2	2.5	3.0
	Mature (5 d)	1.9	2.2	2.3
	Senescent (12 d)	1.8	1.7	1.5
Chl (*a*+*b*)/Car	Young (2 d)	2.3	3.9	4.2
	Mature (5 d)	5.7	5.2	5.1
	Senescent (12 d)	4.8	2.3	1.4

During the maturity of leaves (5th day), the Chl *a*/*b* ratio was 1.9 in MI, 2.2 in HI-1, and 2.3 in HI-2 seedlings (Table 1). However, the ratios declined to 1.8, 1.7, and 1.5 in the MI, HI-1, and HI-2 leaves, respectively, during the senescence phase (12 d). The Chl/Car ratios (Table 1) during mature stage of primary leaves were around 5.0 in HI leaves compared to 5.7 in MI leaves. The ratio declined much faster in HI samples than in the MI ones, reaching 2.3 in HI-1 and 1.4 in HI-2. Thus the degradation of Chl in HI plants was relatively faster than in the MI ones.

The protein content in HI-2 sample was low compared to control (MI) and HI-1 (Fig. 2). Contrary to the slower rate of loss of the macromolecule from 5 d

onward in HI-2, the rate of loss was much faster in the control (from 7 d) and HI-1 (from 5 d) samples.

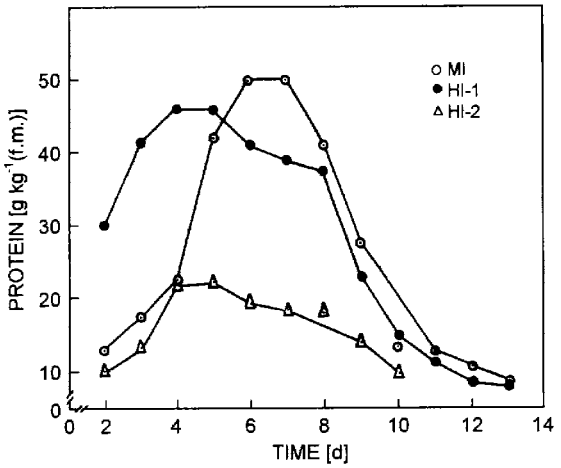

Fig. 2. Protein content of primary leaves of wheat seedlings grown in laboratory under different irradiances.

Table 2. Room temperature chlorophyll (Chl) fluorescence intensity of chloroplasts isolated from primary leaves of wheat seedlings grown under moderate (MI) and high (HI-1 and HI-2) irradiances. The samples were excited at 450 nm. For other details, see Materials and methods.

	Time [d]	F_{685}	F_{735}	F_{685}/F_{735}
MI	3	26.66	3.76	7.09
	5	36.77	5.10	7.21
	7	51.89	7.01	7.40
	9	56.37	7.38	7.63
	11	60.86	7.75	7.85
	13	51.23	6.08	8.40
	15	48.15	5.75	8.37
HI-1	3	51.15	6.65	7.69
	5	58.14	8.05	7.22
	7	65.62	8.08	8.12
	9	73.57	7.25	10.14
	11	45.46	4.17	10.90
	13	30.30	3.49	8.69
HI-2	3	62.23	7.30	8.66
	5	68.01	7.85	8.66
	7	72.80	8.40	8.66
	9	68.94	8.83	10.09
	11	64.74	6.12	10.59

As concerns Chl fluorescence of leaves of seedlings grown under different irradiance (Table 2), a gradual increase in F_{685} continued up to 11 d in MI, to 9 d in HI-1, and to 7 d in HI-2 seedlings followed always by a decline. Similar trends of increase and decrease were also noted in F_{735} values in control sample. However, the decline in the intensity of F_{735} started from 7 d in HI-1 and HI-2 samples. The ratio (F_{685}/F_{735}) in MI leaves increased throughout seedling growth except for 13 d. On the other hand, the ratios in HI-1 and HI-2 plants oscillated in a similar extent.

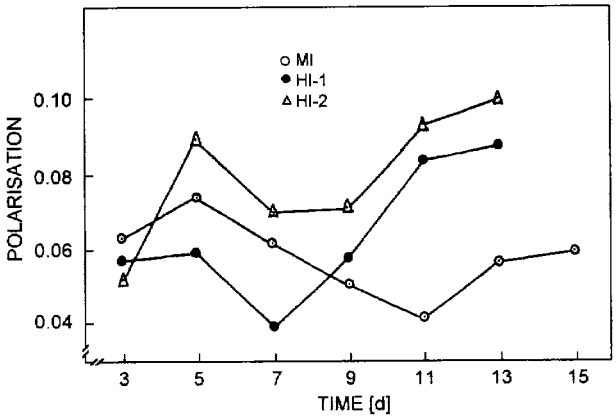

Fig. 3. Membrane polarisation of chloroplasts isolated from the primary leaves of wheat seedlings grown in laboratory under different irradiances. The samples were excited at 620 nm and the emission was recorded at 685 nm.

A triphasic kinetic of changes in polarisation was observed in all the three samples (Fig. 3). It increased initially for 48 h, declined rapidly, and was followed by a new increase. However, except the initial (3 d) value, polarisation of chloroplasts of HI-2 plants was higher than in HI-1 and MI samples during the entire period of seedling growth.

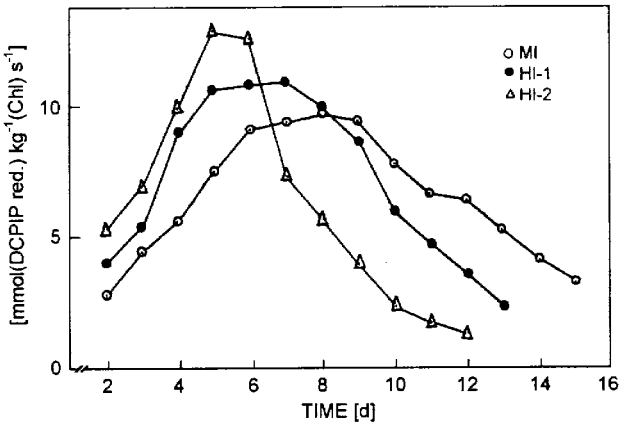

Fig. 4. DCPIP-photoreduction of chloroplasts isolated from primary leaves of wheat seedlings grown in laboratory under different irradiances.

Hill DCPIP activity of chloroplasts isolated from HI-1 and HI-2 leaves was higher and the peak activity was reached earlier (Fig. 4) than in the control (MI). The activity declined after 6, 7, and 9 d in HI-2, HI-1, and MI, respectively. The kinetics of Hill activity matched with total Chl content (Fig. 1) of all types of seedlings.

Discussion

The developmental status of an organ (such as an expanding leaf) is given by its pigment and protein contents and the photochemical activities that increase with age and remain stable (mature stage) followed by decline (senescence). Pigment analyses of primary wheat leaves grown under MI, HI-1, and HI-2 (Fig. 1) suggest that HI causes faster rates of accumulation and loss of Chl (Lichtenthaler *et al.* 1981, 1982, 1984). There was also difference in pigment composition in the leaves as indicated from the pigment ratios (Table 1). The higher Chl *a/b* ratio during mature stage in HI leaves indicates low LHC content in chloroplasts (Prenzel *et al.* 1980). On the other hand, low Chl (*a+b*)/Car ratio suggested relatively more Car in the leaves. Since more photons are available for the HI plants, the leaves have adapted to HI by having less light-harvesting (Chl-*b*) and more photoprotecting (Car) pigments (Table 1).

One of the causes of early loss of Chl in HI-1 and HI-2 is the photooxidation of pigments (Powles 1984). Excess irradiance causes formation of various reactive oxygen species through triplet Chl (Young 1991) and due to impaired electron transport in chloroplasts (Alscher *et al.* 1997, Biswal and Biswal 1999). These highly reactive substances are responsible for faster degradation of Chl. The rates of accumulation and loss of Car were also faster in the HI-1 and HI-2 samples. At HI, Cars are synthesised at a higher rate in the leaves (Bilger and Björkman 1990, Schindler *et al.* 1994). This is a strategic adaptation of plants: if plants are subsequently exposed to HI, the Car protect them from the irradiance stress (Choudhury *et al.* 1993, 1994, Gilmore 1997, Minkov *et al.* 1999). As the yellow pigment gets itself degraded on quenching the harmful triplet Chl and reactive oxygen species formed under excess irradiance (Panda and Biswal 1989, Choudhury and Choe 1996), the loss was more in HI-1 and HI-2 compared to control.

HI plants contain less protein (Arntzen 1978). One of the reasons of this is lower amount of LHCP in HI leaves (Meier and Lichtenthaler 1981, Lichtenthaler *et al.* 1982). The low content of proteins in the HI-2 sample (Fig. 3) could also be due to high rate of breakdown caused by photon stimulation of protease activity (Andersson and Aro 1997, Paulsen 1997).

Information on coupling of photon absorption and photochemical reactions is obtained by monitoring fluorescence characteristics of chloroplasts (Krause and Weis 1991). A gradual increase in Chl fluorescence (F_{685}) intensity during seedling growth (Table 2) suggests changes in thylakoid organisation. However, the differential increase in the ratio F_{685}/F_{735} in MI and HI-1 samples may suggest a different temporal kinetics of development of the two photosystems. On the other hand, the ratio in HI-2 plants remained stable up to 7 d suggesting similar kinetics of structural development of the two photosystems during leaf growth.

Sharp increase in fluorescence polarisation (Fig. 3) particularly during the senescence suggests an ageing-induced conversion of thylakoid membrane to gel phase (Panda and Biswal 1989, Behera and Choudhury 1997). This may hinder charge separation in the Chl molecules leading to decline in photochemistry of chloroplasts (Fig. 4). Polarisation increased more in HI-1 and HI-2 plants than in the control. This may also suggest uncoupling of LHC and reaction centre of photosystems resulting in low photochemisry during senescence of leaves (Swain *et al.* 1990, Behera and Choudhury 1997).

The DCPIP photoreduction activity of isolated chloroplasts reflects the photochemical potential of PS2. During leaf development, the highest pigment contents coincide with the maximum photochemical activity of chloroplasts. Chloroplasts isolated from MI, HI-1, and HI-2 leaves showed highest Hill activity (Fig. 4) which coincided with the maximum Chl content (Fig. 1). The decline in photochemistry also coincided with the loss in pigment contents. The high rate of photochemistry in HI-1 and HI-2 samples could not be maintained for longer period as observed in the control and declined sooner because of loss of pigments as well as HI induced changes in the microenvironment of thylakoid membrane. These observations suggest that wheat seedlings grown under different irradiances have different leaf pigment composition and photochemical potential of the chloroplasts.

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