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High irradiance induced pigment degradation and loss of photochemical activity of wheat chloroplasts

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Abstract

Loss of chlorophyll (Chl) and carotenoids (Car) of leaves and changes in Chl fluorescence emission and polarisation, malondialdehyde (MDA) accumulation, and 2,6-dichlorophenol indophenol (DCPIP) photoreduction in chloroplasts of wheat seedlings grown under different irradiance and subsequently exposed to high irradiance stress (HIS; 250 W m^{-2}) were studied in mature and senescent primary wheat leaves. Faster rate of pigment loss was observed in leaves of moderate irradiance (MI; 15 W m^{-2}) grown plants, compared to high irradiance (HI-1 and HI-2; 30 and 45 W m^{-2}) ones when exposed to HIS. A relatively lower loss of Car in the plants grown in HI-1 and HI-2 exposed to HIS suggests HI adaptation of these seedlings. The slower rate of increase in the ratio of Chl fluorescence emission (F_{685}/F_{735}) also may suggest photoprotective strategy of HI grown seedlings. There was a positive correlation between MDA accumulation and Chl fluorescence polarisation. The DCPIP photoreduction activity in chloroplasts isolated from HI-1 and HI-2 grown plants exposed to HIS showed slower loss of electron transport activity compared to MI grown plants. These observations suggest that plants grown under higher irradiance have capacity to manage the excess quanta better than those grown under lower irradiance.

Additional key words: carotenoids, chlorophyll, 2,6-dichlorophenol indophenol reduction, fluorescence, leaf age, malondialdehyde, photoinhibition, senescence.

Introduction

Carotenoids (Car) serve at least two important functions during photosynthesis, namely radiant energy harvesting and photoprotection of chloroplasts. They prevent the formation of highly destructive reactive oxygen species by intercepting the triplet-chlorophyll (Siefermann-Harms 1987, Young 1991). Car also act as light harvesting pigments by absorbing photons and transferring the excitation energy to chlorophyll (Chl) which eventually reaches the reaction centre.

A considerable part of the radiation absorbed by pigments of green plants is reemitted back as fluorescence. Balancing the amount of photons absorbed and its chanelisation through chloroplasts is one of the major challenges faced by plants. A number of regulatory processes occur in the chloroplasts modulating the efficiency of photon capture and its utilisation (Choudhury and Choe 1996, Genty and Harbinson 1996, Horton *et al.* 1996). Studies of photoinhibition of photosynthesis have revealed that the photosystem 2 (PS 2) activity is effectively regulated by the plants (Giardi *et al.* 1997). This is done by inactivation of PS 2 when radiation exceeds the level plastid can process. The primary target of high irradiance induced damage is the PS 2 reaction centre, where electron transfer between the primary electron doner, Tyr-Z_1 and the secondary plastoquinone acceptor, Q_B, occurs (Barber *et al.* 1997, Giardi *et al.* 1997).

In the present work, effect of high irradiance stress (HIS) was investigated on wheat seedlings grown under different irradiances and results are analysed to ascertain if the leaves develop any strategy to counteract irradiance stress.

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Abbreviations: Car - carotenoids; Chl - chlorophyll; DCPIP - 2,6-dichlorophenol indophenol; HI - high irradiance; HIS - high irradiance stress; MDA - malondialdehyde; MI - moderate irradiance; PS 2 - photosystem 2.

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Materials and methods

Plants: Wheat (*Triticum aestivum* L. cv. Sonalika) seedlings were grown in a culture room on sterilised cotton in Petri dishes at temperature of 25 ± 2 °C and continuous "white light". The irradiance was different: moderate irradiance (MI, 15 W m^{-2}) and high irradiance (HI-1, 30 W m^{-2} and HI-2, 45 W m^{-2}). The primary leaves were harvested at 24 h intervals for various experiments during mature and senescence phases. Wheat seedlings 5-d-old (grown under HI-1 and HI-2) and 7-d-old (grown under MI) were exposed to HIS at 250 W m^{-2} for 4 h daily for the next 5 d. Leaf samples were taken just after HI treatment and used for pigment analysis and isolation of chloroplasts for measurement of fluorescence emission, and polarisation, malondialdehyde (MDA) accumulation, and photochemical activity.

Pigment estimation: The pigments were extracted in 80 % chilled acetone. The amount of total Chl and Car were estimated spectrophotometrically (*UV-150-02, Shimadzu*, Tokyo, Japan) according to Lichtenthaler (1987).

Chloroplast isolation: About 25 leaves were homogenised in pre-chilled mortar and pestle with ice-cold isolation medium containing 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 cheese cloth and the filtrate was centrifuged at 500 *g* for 1 min. The supernatant was again centrifuged at 1000 *g* for 10 min, and the pellet was collected in a small volume of homogenising medium.

Measurement of Chl fluorescence and polarisation: The room temperature Chl *a* fluorescence emission of chloroplasts was measured by a spectrofluorometer (model *650-40, Hitachi*, Tokyo, Japan) following the procedure of Swain *et al.* (1990). For scanning a slit of 5 nm width was used. 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 suspension medium were taken for all experiments.

For measuring the fluorescence polarisation, the chloroplast suspension was excited at 620 nm and polarisation was recorded at 685 nm. Polarisation was calculated according to the formula of Swain *et al.* (1990) with little modification.

Measurement of MDA accumulation: The amount of MDA, a product of thylakoid lipid peroxidation, was estimated according to Du and Bramlage (1992). 0.5 % thiobarbituric acid in 20 % trichloroacetic acid was added to an equal volume of chloroplast suspension (10 μg(Chl) cm^{-3}) along with a few grains of acid washed sand. The suspension was kept in a water bath at 95 °C for 25 min and then centrifuged at 3000 *g* for 5 min for clarification. Absorbance of the clear solution was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The amount of accumulated MDA was estimated by using an absorption coefficient of 155 mM cm^{-3}.

Measurement of photochemical activities: The 2,6-dichlorophenol indophenol (DCPIP) photoreduction by isolated chloroplasts was measured spectrophotometrically as described by Swain *et al.* (1990). The 3 cm^3 reaction mixture contained chloroplasts equivalent to 15 μg 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 minimize infra-red radiation. The photoreduction of the DCPIP was measured at 600 nm.

Results and discussion

Chl content of primary leaves decreased during leaf ontogeny in dependence on growing irradiance. The decrease in Chl content in mature primary leaves of the seedlings grown under MI was 25 % of the initial, while decrease in Chl content in mature leaves of HI-1 and HI-2 seedlings was 65 and 71 %, respectively (Fig. 1*A*). However, when the MI seedlings were exposed to HIS, the loss of pigment was 58 % after 5 d of stress. This indicated a 33 % increase in pigment loss when the MI seedlings were exposed to HIS. The corresponding increases in the pigment loss in the HI-1 and HI-2 seedlings on HIS exposure were 23 and 10 %, respectively. This indicates that the plants grown under higher irradiance when subjected to HIS suffer relatively less pigment degradation compared to those grown at MI.

Plants exposed to higher irradiance accumulate pigments and macromolecules faster and reach mature stage earlier compared to the seedlings grown under lower irradiance (Lichtenthaler *et al.* 1981, Powles 1984, Behera and Choudhury 2001). The phase of decline (senescence) is also initiated earlier in the seedlings grown under higher irradiance. However, when the plants grown under HI are exposed to HIS, the photo-oxidative degradation of pigment is less compared to the plants grown under low irradiance (Lichtenthaler *et al.* 1982, Powles 1984, Ivanov *et al.* 1998).

Similar decrease in Car, *i.e.*, 11, 37 and 60 % in MI, HI-1, and HI-2 seedlings, was found in mature primary

leaves (Fig. 1*B*). However, when the seedlings were exposed to HIS, higher rate of Car loss was observed in MI grown seedlings (41 %) compared to HI-1 (18 %) and HI-2 (8 %) ones. Car protect Chl from photooxidative degradation (Siefermann-Harms 1987, Choudhury *et al.* 1993, 1994). During the protective action, they themselves get degraded. However, plants grown under higher irradiance accumulate more Car compared to the seedlings grown under lower irradiance (Thayer and Björkman 1990, Long and Humphries 1994, Schiefthaler *et al.* 1999). The higher Car content in seedlings protects the photosynthetic machinery if subsequently exposed to radiation stress. This is why pigment loss is less when HI-1 and HI-2 grown seedlings are exposed to HIS compared to MI plants.

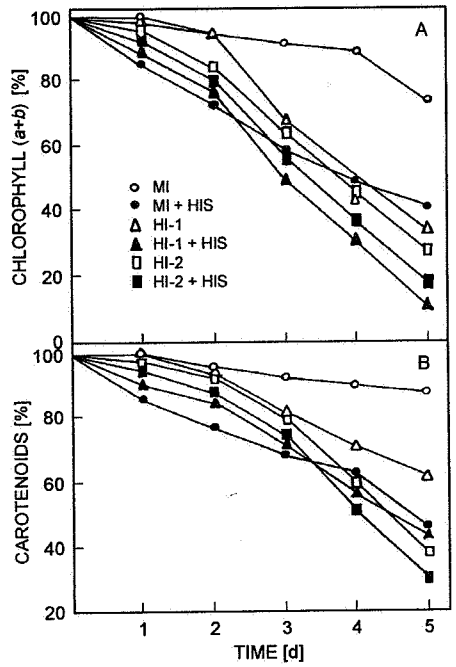

Fig. 1. Chlorophyll (*a*+*b*) (*A*) and carotenoid (*B*) contents of primary leaves of wheat seedlings grown in laboratory under different irradiances and exposed to high irradiance stress (HIS) after the primary leaves reached mature stage. The initial values for chlorophylls and carotenoids of 100 % at zero day were equal to [g kg^{-1}(f.m.)] 2.07 and 0.36 for medium irradiance (MI), 3.6 and 0.68 for high irradiance (HI-1), and 3.96 and 0.77 for HI-2.

Table 1. Chl *a* fluorescence emission of chloroplasts isolated from primary leaves of wheat seedlings grown under medium (MI) or high (HI-1, HI-2) irradiances and subsequently exposed to high irradiance stress (HIS).

Treatments	Time [d]	F_{685}	F_{735}	F_{685}/F_{735}
MI	0	51.89	7.01	7.40
	5	51.23	6.08	8.42
MI + HIS	5	75.03	8.00	9.37
HI-1	0	58.14	8.05	7.22
	5	45.46	4.17	10.90
HI-1 + HIS	5	97.76	8.55	11.43
HI-2	0	68.01	7.85	8.66
	5	64.74	6.12	10.57
HI-2 + HIS	5	105.00	9.59	10.94

Chl fluorescence emission spectra of a green leaf measured at room temperature exhibit usually two emission maxima, the first at 685 nm and the second at 735 nm. Changes in the ratio of F_{685}/F_{735} under stress inform about the photochemistry in photosynthesis (Lichtenthaler 1996). The higher increase of Chl

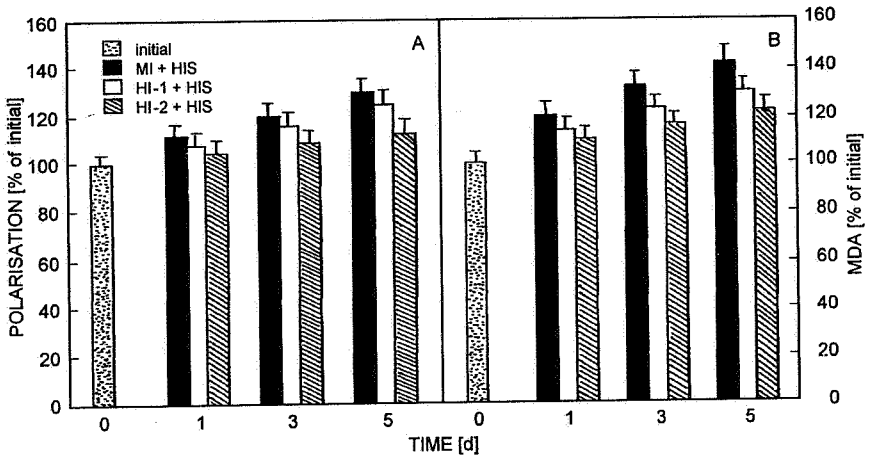

Fig. 2. Membrane polarisation (*A*) and thylakoid lipid peroxidation (*B*) of chloroplasts of primary leaves of wheat seedlings grown in laboratory under different irradiances and exposed to high irradiance stress (HIS) after the primary leaves reached maturity. The initial values of 100 % for polarisation [relative] and lipid peroxidation [μmol(MDA) g^{-1} (f.m.)] at zero day were equal to 0.062 and 0.54 for medium irradiance (MI), 0.06 and 0.56 for high irradiance (HI-1), and 0.09 and 0.59 for HI-2, respectively (*bar* indicates ± SD, $n = 5$).

fluorescence ratio (11.3 %) in MI sample compared to HI-1 (4.8 %) and HI-2 (3.5 %) when exposed to HIS (Table 1) may suggest greater effect of radiation stress on the MI sample (Lichtenthaler and Miehe 1997, Gitelson *et al.* 1998).

Measurement of fluorescence polarisation of chloroplasts (Fig. 2*A*) indicated that polarisation increased in all groups of seedlings (MI, HI-1 and HI-2) exposed to HIS. The increase in polarisation was higher in samples from MI plants exposed to HIS compared those from HI-1 and HI-2 plants. The fluorescence polarisation of chloroplasts was positively correlated with MDA accumulation (Fig. 3).

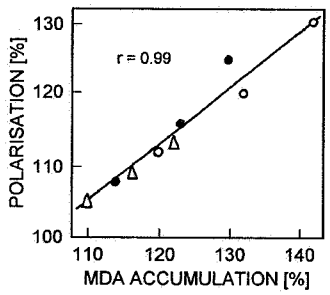

Fig. 3. Correlation between MDA accumulation and fluorescence polarisation of chloroplast of primary leaves of wheat seedlings grown in laboratory under different irradiances and exposed to high irradiance stress (HIS) after the primary leaves reached mature stage. *Open circles* represent medium irradiance (MI), *closed circles* high irradiance (HI-1), and *triangles* HI-2.

Lipid peroxidation increased in all the three types of seedlings exposed to HIS (Fig. 2*B*). The degree of lipid peroxidation, however, was higher in leaves of MI seedlings than in those of HI-1 and HI-2 seedlings. This indicates that plants grown under higher irradiances are less susceptible to HIS (Ivanov *et al.* 1998). Thylakoid lipids are susceptible to fatty acid oxidation due to the predominance of lipids with unsaturated fatty acids (Gounaries *et al.* 1986). Oxygen is always available around functional PS 2 which could be the mediator of free radical reactions (Kyle 1987) and might be involved in the peroxidative degradation of polyunsaturated fatty acyl residues of the thylakoid lipids.

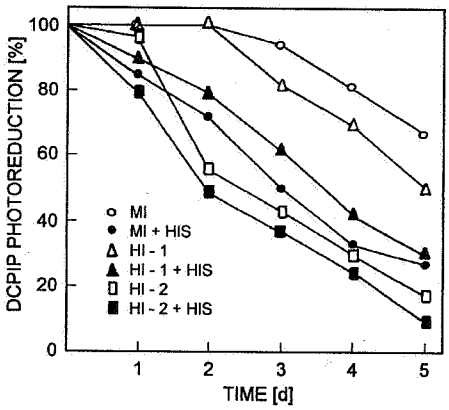

Fig. 4. DCPIP photoreduction of chloroplasts of primary leaves of wheat seedlings grown in laboratory under different irradiances and exposed to high irradiance stress (HIS) after the primary leaves reached mature stage. The initial values of 100 % at zero day were equal to [mmol (DCPIP reduced) kg^{-1}(Chl) s^{-1}] 9.44 for medium irradiance (MI), 10.27 for high irradiance (HI-1), and 13.05 for HI-2.

The loss of electron transport activity of chloroplasts, in terms of DCPIP photoreduction, was maximum (40 %) in MI plants when exposed to HIS compared to 20 and 8 % losses in HI-1 and HI-2 plants, respectively (Fig. 4). This decrease in electron transport activity of chloroplasts under HIS suggests occurrence of photoinhibition. The decrease in activity is due to degradation of D1 protein (Long and Humphries 1994, Barber *et al.* 1997). The leaves can protect the photosynthetic machinery from damage through various mechanisms (Harbinson 1994, Genty and Harbinson 1996). Above mentioned lower decrease in DCPIP reduction in chloroplast from HI-1 and HI-2 exposed to HIS suggests that plants grown under HI are better equipped to handle excess photons (Ivanov *et al.* 1998).

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