Developmental phase-dependent photosynthetic responses to ultraviolet-B radiation: damage, defence, and adaptation of primary leaves of wheat seedlings

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Abstract

Alterations in photosynthetic capacity of primary leaves of wheat seedlings in response to ultraviolet-B (UV-B; 280–320 nm; 60 μmol m^{-2} s^{-1}) exposure alone and in combination with photosynthetically active radiation (PAR; 400–800 nm; 200 μmol m^{-2} s^{-1}) during different phases of leaf growth and development were assessed. UV-B exposure resulted in a phase-dependent differential loss in photosynthetic pigments, photochemical potential, photosystem 2 (PS2) quantum yield, and *in vivo* O_2 evolution. UV-B exposure induced maximum damage to the photosynthetic apparatus during senescence phase of development. The damages were partially alleviated when UV-B exposure was accompanied by PAR. UV-B induced an enhancement in accumulation of flavonoids during all phases of development while it caused a decline in anthocyanin content during senescence. The differential changes in these parameters demonstrated the adaptation ability of leaves to UV-B stress during all phases of development and the ability was modified in UV-B+ PAR exposed samples.

Additional key words: anthocyanin; carotenoids; chlorophyll; flavonoids; O_2 evolution; photosynthetically active radiation; phases of leaf development; senescence; thylakoid membrane.

Introduction

Ultraviolet-B (UV-B) radiation in spectral range of 280–320 nm inhibits plant growth and development (Jordan 2002, Zuk-Golaszewska *et al.* 2003, Caldwell *et al.* 2007). This band of radiation reduces the photochemistry of photosystem 2 (PS2) by damaging the photosynthetic apparatus (PSA) at multiple sites including the D1 and D2 reaction centre proteins (Bornman 1989, Strid *et al.* 1990, Jordan 2002, Vass *et al.* 2005, Rodrigues *et al.* 2006, van Rensen *et al.* 2007). It also alters the gene expression (Jordan *et al*. 1994, Rousseaux *et al.* 1999, Brosché and Strid 2003) and ATPase and ribulose-1,5-bisphosphate carboxylase/oxygenase activities (Strid *et al.* 1990, Strid and Anderson 1994), and inactivates violaxanthin de-epoxidase (Pfündel *et al.* 1992).

Plants respond suitably to lessen the UV-B-induced damage of chloroplasts by exhibiting defence mechanisms (Kolb *et al.* 2001, Xiong and Day 2001) and they revamp the damaged apparatus through an efficient mechanism of DNA repair and *de novo* synthesis of UV-B-sensitive PS2 reaction centre proteins (Bornman 1989, Vass 1997) to effectively acclimate to UV-B environment (Wilson and Greenberg 1993, Jansen *et al.* 1998). However, the magnitude of UV-B induced damage of PSA and ability of adaptation to the stress may vary according to the developmental status of the organelle (Teramura and Caldwell 1981, Dillenburg *et al.* 1995). During different phases of growth, leaf exhibits differential responses to stress in general (Biswal *et al*. 2003) and at the transcript level of photosynthetic gene to UV-B stress in particular (Jordan *et al*. 1994, A.-H.-Mackerness *et al*. 1998). Yet a comparative picture of developmental status-dependent UV-B-induced degradation of PSA is hardly available.

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The response of leaves to UV-B exposure in combination with photosynthetically active radiation (PAR) is different from the response to UV-B exposure in the absence of PAR, demonstrating the PAR-mediated alleviation of UV-B induced damage (Adamse and Britz 1992, Kolb *et al.* 2001, Bergo *et al.* 2003, Pradhan *et al.* 2006). Similar protective role of low irradiance by "white light" against UV-B induced impairment in photosynthetic apparatus of cyanobacterium *Spirulina platensis* was also found (Rajagopal *et al.* 2005). The redox status of PS2 controls the degree of UV-B-induced degradation of D1 and D2 proteins under the combined irradiation by PAR and UV-B (Babu *et al.* 1999). Thus the organization status of the PSA determines the nature and degree of UV-B-induced damage which could be modulated by UV-B+PAR irradiation. Therefore we studied the response of primary leaves of wheat seedlings during different stages of their development to UV-B radiation in the presence and absence of PAR by comparing the damage to the apparatus and the degree of adaptation.

Materials and methods

Wheat (*Triticum aestivum* L. cv. Sonalika) seedlings were grown in Petri plates on cotton soaked with distilled water under fluorescent "white light" of 200 μmol m^{-2} s^{-1} at 25±2 °C following the method of Joshi *et al.* (1993) for a period of 15 d. One set of seedlings were exposed to UV-B radiation (PPFD 60 μmol m^{-2} s^{-1} obtained from a *Philips TL 20* type *05* in the spectral range 280–320 nm and with a peak at 315 nm; without UV-A and UV-C components) in the absence of PAR (UV-B) while another set of seedlings were exposed to UV-B radiation in combination with PAR (UV-B+PAR) for 1 h daily from d 1 until d 15 as per Pradhan *et al.* (2006). The primary leaves of these seedlings were used for various measurements. All biochemical and biophysical measurements were conducted within 1-h UV-treatments.

Contents of total chlorophyll (Chl) and total carotenoids (Car) in the primary leaves of wheat seedlings were determined according to Wellburn and Lichtenthaler (1984). TCA-insoluble total leaf protein content was estimated from the residue following the method of Lowry *et al.* (1951).

Chl fluorescence parameters were measured from attached primary leaves of wheat seedlings with pulse amplitude modulated (PAM) fluorometer *FMS 1* (*Hansatech Instruments*, UK) according to the protocol followed by Schreiber *et al.* (1986). The samples were dark adapted for 10 min and exposed to a saturating pulse for fluorescence measurement. The leaves were then adapted to "actinic light" source of 1 500 μmol m^{-2} s^{-1} and fluorescence parameters of irradiation-adapted leaves were measured. Fluorescence decline ratio (R_{Fd}), the vitality index of leaf, was calculated from the fluorescence parameters following the formula $R_{Fd} = (F_p - F_s)/F_s$.

Photosynthetic oxygen evolution was measured directly from the leaves with a leaf disk electrode unit *LD 2/3*, *Leaf lab 2* (*Hansatech Instruments*, King's Lynn, UK) at 25 °C and at saturating CO_2 conditions.

Chloroplasts were isolated from primary leaves of wheat seedlings following the method of Izawa and Good (1968) and suspended in a medium containing 300 mM sucrose, 50 mM NaCl, and 50 mM Na/K phosphate buffer (pH 6.9). The Chl concentration of the chloroplasts suspension was 2 mg cm^{-3}.

Absorption spectra of isolated chloroplasts were measured with a UV-visible spectrophotometer. Room temperature excitation and emission fluorescence spectra of isolated chloroplasts were measured with a *Shimadzu RF 5000* spectrofluorimeter following the method of Panda *et al.* (1987). Chloroplasts equivalent to 5 μg(Chl) cm^{-3} in the basic assay buffer were excited at 620 nm and the emission was monitored at 685 nm. The excitation spectra were measured within excitation range 400–600 nm for emission at 685 nm.

Accumulation of flavonoids in the primary leaf of wheat seedlings was estimated following Flint *et al.* (1985). A known mass of leaf tissue was boiled vigorously for 10 min in 5 cm^3 of a mixture of ethanol and acetic acid (99 : 1). The final volume of the extract was adjusted to 5 cm^3 and absorption was measured at 270 nm.

Anthocyanin accumulation in the primary leaf of wheat seedlings was estimated according to Beggs and Wellmann (1994). A known mass of leaf tissue was taken in 5 cm^3 of ethanol : HCl (100 : 1) mixture and kept in darkness for 24 h. Absorbance of the extract was measured at 546 nm.

Student's *t*-test was carried out according to Glantz (1989) for statistical analysis.

Results

Different phases of development of primary leaf of wheat seedlings were characterized on the basis of contents of total Chl and total leaf protein (Fig. 1). The pigment and protein contents in primary leaf of wheat seedlings increased up to d 7 (developing phase), remained stable till d 11 (steady phase), and gradually declined thereafter (senescing phase). On d 15 the total Chl and total leaf protein declined by 44 and 42 % of their steady state values, respectively.

The contents of Chl (Fig. 2*A*) and Car (Fig. 2*B*) declined upon UV-B exposure throughout the leaf ontogeny, but the rates of decline during different phases of growth and development were different. The decline of Chl content was 16.49 % (p>0.005), 25.25 % (p>0.005),

Table 1. Changes in contents of chlorophyll/carotenoids (Chl/Car), position of red peak of absorption spectra, Car-to-Chl energy transfer efficiency (E_{475}/E_{600}), photosystem 2 (PS2) fluorescence at 685 nm (F_{685}), and ratio of R_{Fd} to O_2 evolution (R_{Fd}/O_2) from primary leaves of wheat seedlings exposed to UV-B and UV-B+PAR. For details see Materials and methods. Means of three independent measurements, $n = 3$. Significance: $^{*}0.01$; $^{**}0.05$; $^{***}0.005$.

Sample	Treatment	Chl/Car [%]	Red peak position [nm]	E_{475}/E_{600} [%]	F_{685} [%]	R_{Fd}/O_2
7 d old	Control	100.0	678	100.0	100	1.000
	UV-B	115.3***	679.5	82.5**	95	1.053
	UV-B+ PAR	107.8***	678	73.0***	106	1.053
11 d old	Control	100.0	678	100.0	100	1.000
	UV-B	116.5***	680	91.0**	98	1.042
	UV-B +PAR	111.2***	679	120.0***	87	0.794*
15 d old	Control	100.0	676	100.0	100	1.000
	UV-B	88.5***	670	95.0**	116	0.250***
	UV-B+PAR	85.8***	676	85.0**	116	0.345***

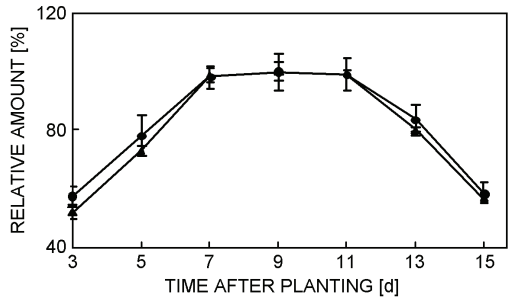

Fig. 1. Different phases of development of primary leaves of wheat seedlings were characterized on the basis of changes in the total contents of chlorophyll (Chl) and leaf protein. 100 % of Chl (●) and protein (▲) were 2.87 and 17.81 [g kg^{-1}(FM)], respectively. $n = 3$. *Bars* indicate ±S.D.

and 22.00 % (p>0.01) in UV-B, and 2.00 % (p>0.1), 4.00 % (p>0.01), and 18.30 % (p>0.02) in UV-B+PAR exposed samples on d 7, 11, and 15, respectively. Similarly the content of Car on d 7, 11, and 15 declined by 27.45 % (p>0.05), 35.80 % (p>0.001), and 17.80 % (p>0.02) in UV-B exposed samples, respectively. In UV-B+PAR exposed sample, the content of Car declined by 8.94 % (p>0.5), 13.90 % (p>0.01), and 7.34 % (p>0.01) on d 7, 11, and 15, respectively.

The Chl/Car ratio (Table 1) in response to UV-B exposure increased by 15.3 % (p>0.01) and 16.5 % (p>0.01) on d 7 and 11, respectively, while the ratio declined by 11.5 % (p>0.01) on d 15. In the presence of PAR, the ratio increased by 7.8 % (p>0.01) and 11.2 % (p>0.01) on d 7 and 11, respectively, while it declined by 14.5 % (p>0.01) on d 15.

The ratio of variable to maximum fluorescence (F_v/F_m; dark adapted leaf and F_v'/F_m'; light adapted leaf) (Fig. 2*C,D*) changed differently during different phases of leaf growth in response to UV-B exposure. On d 7, the values of F_v/F_m and F_v'/F_m' diminished marginally by 1.50 % and 0.50 % in UV-B and by 0.50 % and 0.10 % in UV-B+PAR exposed samples, respectively. But the decline in F_v/F_m and F_v'/F_m' in UV -B exposed leaves was 2.50 % (p>0.05), 5.57 % (p>0.5) on d 11 and 27.50 % (p>0.001) and 14.98 % (p>0.1) on d 15, respectively. On the other hand, on d 15 the ratios F_v/F_m and F_v'/F_m' in UV-B+PAR exposed sample were higher than their corresponding control values by 4.50 % (p>0.01) and 1.80 % (p>0.1), respectively.

Fig. 2*E–H* depicts changes in PS2 quantum yield (Φ_{PS2}), fluorescence decline ratio (R_{Fd}), photochemical quenching (q_p), and non-photochemical quenching (NPQ) in response to UV-B exposure alone or in combination with PAR. Φ_{PS2} declined by 1.0 % (p>0.1), 20.0 % (p>0.005), and 33.0 % (p>0.005) on d 7, 11, and 15, respectively in UV-B exposed seedlings (Fig. 2*E*). On the other hand, a loss of 24.5 % (p>0.0005) and 9.0 % (p>0.1) in Φ_{PS2} on d 11 and 15, respectively, without affecting the parameter on d 7 was observed in UV-B+ PAR exposed sample.

In UV-B exposed seedlings, R_{Fd} value declined by 2 % (p>0.5), 56 % (p>0.005), and 89 % (p>0.005) (Fig. 2*F*) of their corresponding control on d 7, 11, and 15, respectively. Similarly, in UV-B+PAR exposed leaves the values of R_{fd} changed marginally on d 7 while it declined by 24 % (p>0.005) and 58 % (p>0.005) of their corresponding control on d 11 and 15, respectively.

Photochemical quenching (q_p) (Fig. 2*G*) was enhanced marginally by 2.0 % in UV-B and 1.5 % in UV-B+PAR exposed sample on d 7. On the contrary on d 11, q_p diminished by 20.0 % (p>0.001) in UV-B and 25.8 % (p>0.001) in UV-B+PAR exposed seedlings (Fig. 2*G*). Similarly on d 15, the parameter diminished by 41.8 % (p>0.001) and 9.7 % (p>0.1) with UV-B and UV-B+PAR exposure, respectively.

NPQ declined by 2.18 % (p>0.5) and 48.70 % (p>0.001) on d 7 and 15, respectively, while on d 11 it was enhanced by 31.90 % (p>0.1) in UV-B exposed sample (Fig. 2*H*). On the other hand, in UV-B+PAR exposed sample the parameter was enhanced by 1.20 % (insignificant) and 78.50 % (p>0.1) on d 7 and 15,

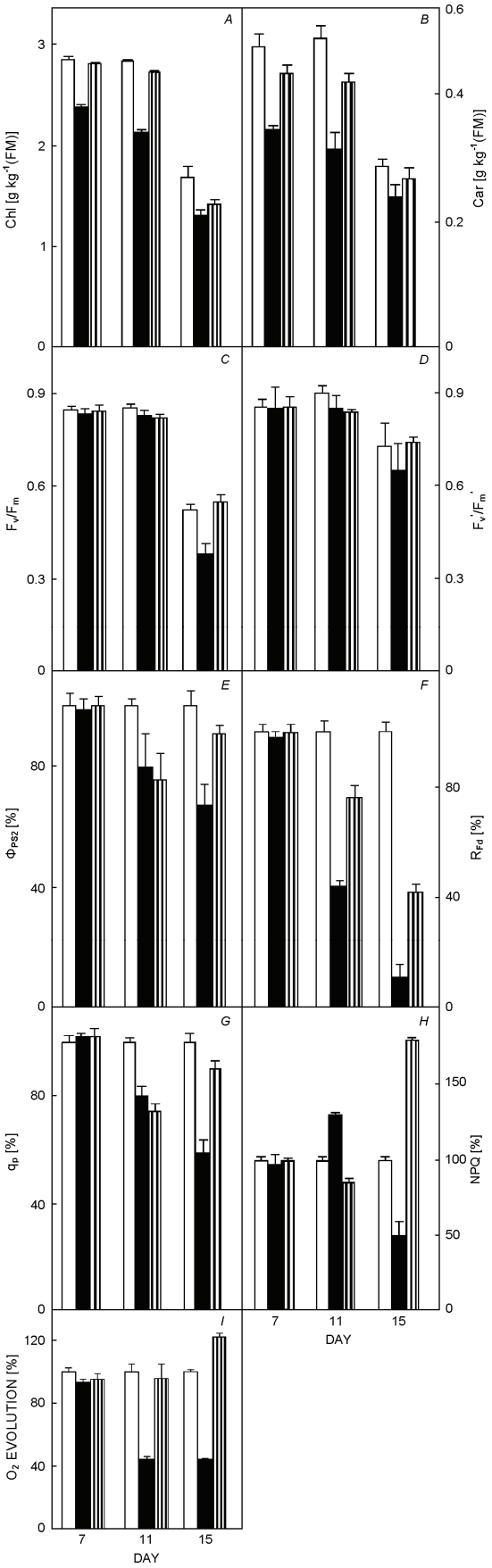

Fig. 2. Changes in contents of (*A*) chlorophyll (Chl) and (*B*) carotenoids (Car), (*C*) F_v/F_m (dark adapted), (*D*) F_v'/F_m' (light adapted), (*E*) Φ_{PS2}, (*F*) R_{Fd}, (*G*) q_p, and (*H*) NPQ, and (*I*) O_2-evolution in primary leaves of wheat seedlings on d 7, 11, and 15 in response to UV-B (*black*) or UV-B+PAR (*vertical strip*) exposure. (*E–I*): The values of Φ_{PS2}, R_{Fd}, q_p, and NPQ of control samples on d 7, 11, and 15 are taken as 100 % of corresponding phases. Control = *white*. *n* = 3 (*A, B, I*) or 6 (*C–H*). *Bars* indicate ±S.D.

respectively while it was diminished by 14.89 % (p>0.5) on d 11 over their corresponding control values.

Rate of O_2-evolution in leaves of UV-B exposed sample declined by 7 % (p>0.5), 56 % (p>0.005), and 56 % (p>0.005) on d 7, 11, and 15, respectively (Fig. 2*I*). On the other hand, in UV-B+PAR exposed sample the rate declined by only 5 % and 4 % on d 7 and 11, respectively. On d 15 the rate of O_2 evolution in UV-B+PAR exposed sample was, however, higher than that of the control.

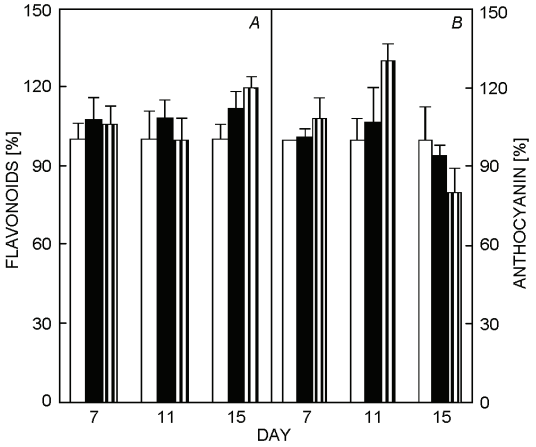

Fig. 3. Relative changes in contents of flavonoids (*A*) and anthocyanin (*B*) in primary leaves of wheat seedlings on d 7, 11, and 15 in response to UV-B (black) or UV-B+PAR (vertical strip) exposure. Control = white. The values of these parameters of control samples on d 7, 11, and 15 are taken as 100 % of corresponding phases. *n* = 3. *Bars* indicate ±S.D.

The position of red-peak in absorption spectra, energy transfer efficiency, and peak height of room temperature fluorescence at 685 nm of isolated chloroplasts (Table 1) were changed on UV-B exposure. The position of red peak was red-shifted by 1.5 and 2.0 nm on d 7 and 11, respectively, but blue-shifted by 6.0 nm on d 15 in response to UV-B exposure. These shifts were not observed in UV-B+PAR exposed samples. Similarly, in response to UV-B exposure the Car-to-Chl energy transfer efficiency calculated from fluorescence excitation intensities on d 7, 11, and 15 of isolated chloroplasts declined by 17.5, 9.0, and 5.0 %, respectively. In UV-B+PAR exposed seedlings the efficiency was further suppressed by 10 % on both d 7 and 15, but was enhanced by 20 % on d 11.

The fluorescence emission intensity (F_{685}) declined marginally on d 7 and 11 but increased by 16 % on d 15 in response to UV-B exposure. On the other hand, the UV-B+PAR exposure enhanced it by 6 and 16 % on d 7 and 15, respectively. On d 11 the decline was furthered by 11 % in UV-B+PAR exposed sample.

The accumulations of flavonoids (Fig. 3*A*) and anthocyanin (Fig. 3*B*) in the primary leaf of wheat seedlings were altered differently in response to UV-B exposure in the presence or absence of PAR. The content of flavonoids was enhanced by 7.6 % (p>0.5), 8.0 % (p>0.1), and 11.7 % (p>0.05) on d 7, 11, and 15, respectively, in UV-B exposed seedlings. The accumulation of flavonoids was enhanced by 5.6 % (p>0.5) on d 7 and 19.0 % (p>0.05) on d 15 but on d 11 the content was almost equal to that of control in UV-B+PAR exposed sample.

The content of anthocyanin was increased by 6.5 % (p>0.01) on d 11 while it decreased by 6.1 % (p>0.01) on d 15 in response to UV-B exposure. On the other hand, in UV-B+PAR exposed sample, an increase in the accumulation of anthocyanin by 8.3 % (p>0.02) on d 7 and 30.0 % (p>0.01) on d 11 was observed while on d 15 its content declined by 20.0 % (p>0.05).

Discussion

The patterns of changes in the contents of total Chl and total leaf protein in the primary leaf of wheat seedlings (Fig. 1) exhibited the occurrence of developing phase up to d 7 followed by steady phase till d 11 and declining senescence phase thereafter. On d 15 the content of total Chl and total leaf protein declined by nearly 50 % of their steady state values. Therefore, different parameters have been measured on d 7, 11, and 15 to assess the degree of UV-B induced changes during developing, steady, and senescence phases of leaf growth, respectively.

UV-B exposure resulted in a loss in Chl (Fig. 2*A*) and Car (Fig. 2*B*) contents during all phases of leaf growth but the extent of loss was different in different phases. From the pattern of changes in Chl and Car (Fig. 2*A,B*) and Chl/Car (Table 1), preferential damage of light-harvesting Chl-protein complex (LHCP) during developing and steady phases and uniform loss in both peripheral and reaction centre components of PSA during senescing phase in response to UV-B exposure have been inferred. The loss in the photosynthetic pigments (Table 1) is attributed to membrane disorganization as revealed by the changes in the position of red peak in room temperature absorption spectra and room temperature Chl fluorescence intensity (Panda *et al*. 1987, Biswal *et al.* 2003). UV-B mediated decline in Car-to-Chl energy transfer efficiency (Table 1) suggests that Car proximity to Chl is lost in the disorganized membrane. Consequently, Cars fail to quench harmful triplet Chl leading to a faster degradation of the membrane. Further, the comparison of these results shows that the PSA is susceptible to UV-B radiation throughout the leaf ontogeny but the damage during senescing phase is larger than that during developing and steady phases of leaf growth. On the other hand, in UV-B+PAR exposed samples, loss in photosynthetic pigments is relatively less than in UV-B exposed ones. Critical analyses of the results of Chl/Car ratio, position of absorption peak, fluorescence intensity, and Car-to-Chl energy transfer efficiency (Table 1) indicated an improved status of thylakoid membrane in the UV-B+ PAR exposed sample as compared to that of UV-B exposed one.

Analysis of PAM fluorescence provides a comprehensive insight into the primary photochemistry, PS2 quantum yield, and oxidative damages of PSA (Lichtenthaler 1990, van Kooten and Snel 1990, Govindjee 1995, Strasser *et al.* 2004, van Rensen *et al.* 2007) while the rate of O_2 evolution is an indicator of net photosynthesis. UV-B-induced changes in F_v/F_m, F_v'/F_m' (Fig. 2*C,D*), Φ_{PS2}, R_{Fd}, q_p, NPQ (Fig. 2*E–H*), and O_2 evolution (Fig. 2*I*) on d 7 are marginal and suggest that the young developing leaves are resilient to overcome UV-B hazards. But UV-B exposure induces a significant decrease in F_v/F_m, F_v'/F_m' (Fig. 2*C,D*), q_p, R_{Fd}, and Φ_{PS2} (Fig. 2*H*) on d 11. Comparison of the results of these two phases suggests that leaf during its developing phase of growth and development with all defence mechanisms in active state has enough capacity to recover from UV-B induced damage fully while the capacity to recover from the damage in developed steady leaves is reduced. However, leaves respond to UV-B menace by down-regulating the PS2 efficiency as evidenced from an increase in NPQ during steady state of leaf development. Further, the response of leaves to UV-B radiation in the presence of PAR is different from those in the absence of PAR. The increase in NPQ in spite of enhancement in q_p, F_v/F_m, Φ_{PS2}, and F_v'/F_m' in UV-B+PAR exposed leaves compared to those in UV-B-exposed ones on d 7 revealed that leaf during developing phase regulates the photon energy absorption to protect the PSA from photo-damage through the well-known NPQ mechanism to overcome the UV-B stress.

On the other hand, the decline in F_v/F_m, F_v'/F_m' (Fig. 2*C,D*), Φ_{PS2}, R_{fd}, q_p, NPQ (Fig. 2*E–H*), and *in vivo* O_2 evolution (Fig. 2*I*) in UV-B-exposed leaves on d 15 suggests that the radiation impairs the PSA including the energy dissipation mechanism. In the presence of PAR, the response of senescing leaves to deal with the UV-B peril is significant as indicated by the rise in all these parameters in general and F_v/F_m and NPQ, whose values were even more than those of their corresponding control, in particular. To test if these responses are translated into alteration in functional ability of leaf, the rate of O_2 evolution from leaf segment was compared with R_{Fd}, a fluorescence parameter reflecting the vitality index leaf (Fig. 2*E–H*).

The rate of *in vivo* O_2 evolution is controlled by processes like respiration and CO_2-fixation which in turn are susceptible to UV-B impact and their damage could influence the photosynthetic capacity of leaf. On the other hand, the parameter R_{Fd} correlates to CO_2-fixation (Lichtenthaler 1988, Strasser *et al.* 2004). Therefore the ratio R_{Fd}/O_2 could provide a clue if UV-B-induced alteration in CO_2-fixation has any role in changes of photosynthetic capacity. The ratio in our study (Table 1) was not altered much although R_{Fd} and O_2 evolution (Fig. 2*I*) were diminished significantly because of UV-B exposure on d 7 and 11. But the loss in O_2 evolution was significantly more compared to R_{Fd} on d 15. This finding along with the results of higher loss in pigments and photosynthetic capacity suggests that the impact of UV-B radiation is severe during senescence at both structural and functional level.

On the other hand, the lesser losses in photosynthetic pigments (Fig. 2*A,B*), photochemical potential (Fig. 2*C, D*), Φ_{PS2}, R_{Fd} (Fig. 2*E–H*), and O_2 evolution (Fig. 2*I*) during all phases of leaf growth in UV-B+PAR exposed sample compared to the UV-B exposed one suggest a healthier thylakoid with better functional ability in the former. However, relatively higher loss in O_2 evolution as compared to R_{Fd} (Table 1) on d 11 in UV-B+PAR treated sample cannot be explained at present. UV-B-induced stimulation of photorespiration at the cost of photosynthesis could result in such a decline. Further higher values of F_v/F_m, F_v'/F_m' (Fig. 2*C,D*), R_{Fd}/O_2 evolution ratio (Table 1), and only a small decline in Φ_{PS2} (Fig. 2*E–H*) on d 15 in UV-B+PAR exposed wheat seedlings compared to UV-B exposed ones might suggest an ameliorative effect of PAR and be in agreement with observations of Pradhan *et al.* (2006).

Anthocyanin mitigates the damaging effects on PSA by screening UV-B penetration, while flavonoids, besides their anti-oxidant property, diminish the damaging effect by attenuating UV-B radiation (Holton and Cornish 1995, Mohr and Schopfer 1995, Lau *et al*. 2006). The protection of DNA by anthocyanin and flavonoids was detected by Stapleton and Walbot (1994). Therefore, accumulation of anthocyanin and/or flavonoids in response to UV-B radiation is an adaptive defence mechanism, and their accumulation has been used as a marker of adaptation in our work. UV-B induced enhancement in the contents of flavonoids and anthocyanin in primary leaves of wheat seedlings is in conformity with earlier works (Lindoo and Caldwell 1978, Beggs and Wellmann 1994, Brandt *et al.* 1995, Olsson *et al.* 1998, Gao *et al.* 2004). Accumulation of UV-absorbing substance throughout the leaf development of spinach seedlings has been observed by Hada *et al.* (2001). Therefore the increases in the content of anthocyanin during steady phase and of flavonoids during all phases of development in response to UV-B exposure (Fig. 3) indicate that leaf exhibits adaptation strategy of different extent against UV-B impact during all phases of development although through different mechanism. In UV-B+PAR exposed leaves, the ability of adaptation is further increased.

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Bock, R.: **Cell and Molecular Biology of Plastids**. – Springer, Heidelberg 2007. ISBN 978-3-540-75375-9. 544 pp., 51 figs, 23 tabs. € 259.00, CHF 451.00, USD 339.00, GBP 199.00.

One year after publishing *The Structure and Function of Plastids* [R.R. Wise and J.K. Hoober (ed.)] in the *Advances in Photosynthesis and Respiration* series, Springer is issuing another monograph devoted to plastids, so one cannot avoid comparing them. The first question that emerges is whether there are enough subjects not covered in the first book to justify publication of the second one. Unambiguously, the answer is positive and there is surprisingly little overlap between the two books. On the other hand, this means that the reviewed book cannot be comprehensive as it leaves out some important subjects—such as plastid evolution.

As the title suggests, the emphasis of the book is on molecular aspects of plastid biology. The book begins with the least "molecular" chapter on plastid biogenesis and differentiation written by Kevin Pyke. The second chapter by Ralph Bock covers structure of plastid genomes in plants and algae. Important information here is that not all plants display a maternal mode of plastid inheritance. The following chapter, contributed by Anil Day and Panagiotis Madesis, addresses plastid DNA replication, recombination and repair with emphasis on homologous recombination. The next three chapters provide a detailed view of transcription and its regulation in plastids. The first of these, written by Karsten Liere and Thomas Börner, describes components of the transcriptional apparatus in plastids and their roles in transcription. The second one by David Stern and colleagues deals with transcript maturation and its polyadenylation-stimulated degradation. The role and probable evolutionary history of polyadenylation in plants, cyanobacteria, and chloroplast are also discussed. The third transcriptional chapter by Cristian Schmitz-Linneweber and Alice Barkan is devoted to mRNA intron splicing and editing, acquired traits rarely present in chloroplast bacterial ancestors. Logically, the following five chapters concern proteins. Although the translational mechanism of chloroplast mRNA is similar to that of bacteria, chloroplast ribosomes and translational factors are substantially different and many nuclear-encoded regulatory proteins allow sophisticated translational regulation in response to changing environment, as discussed in the chapter by Hadas Peled-Zehavi and Avihai Danon. However, the majority of chloroplast proteins are nuclear encoded with N-terminal transit sequence and have to be imported into the plastid. The chapter by Birgit Agne and Felix Kessler summarizes the components of the translocation machinery, as well as new developments in the study of alternative import pathways. The assembly of protein complexes in the chloroplast is demonstrated on well-studied photosynthetic complexes by Eva-Mari Aro and her colleagues. The importance of orderly protein degradation in adaptation to changing environmental conditions in the chloroplast is stressed in the chapter written by Zach Adam. The chapter by Bianca Naumann and Michael Hippler gives an excellent overview of the current state of plastid proteomics from methodological approaches to the results of proteomic studies on individual chloroplast compartments. The amazing, quickly developing field of anterograde and retrograde signalling between nucleus and plastid and *vice versa* is summarized in the chapter by Thomas Pfannschmidt and colleagues. Great complexity of the regulatory pathways between prokaryotic and eukaryotic genomes of the plant cell is immediately apparent from their contribution. Lastly, a comprehensive and up-to-date chapter on genetic transformation of plastids written by Hans-Ulrich Koop and colleagues gives a great methodological overview of the available tools and approaches, including resistance markers for targeted gene inactivation and suitable promoters for inducible gene expression.

All in all, this volume gives an excellent overview of the progress and current status of molecular studies on plastids, particularly on chloroplast. The book is recommended for all plant molecular biologists, primarily for those working in plastid biology, photosynthesis, and biotechnology. A complete Table of contents of this book is available at: http://www.springer.com/life+sci/cell+biology/book/978-3-540-75375-9?detailsPage=toc.

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