

Intraspecific responses of six cultivars of wheat (*Triticum aestivum* L.) to supplemental ultraviolet-B radiation under field conditions

Madhu Singh · Suruchi Singh · S. B. Agrawal

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Abstract The solar ultraviolet-B (UV-B) background level is often high and posing an environmental challenge in most of the tropical region of the world, including India. This prompted the present study to investigate the effects of supplemental UV-B (sUV-B) radiation (ambient + $7.2 \text{ kJ m}^{-2} \text{ day}^{-1}$) on various growth, physiological and biochemical characteristics of six locally grown cultivars of wheat (*Triticum aestivum* L.). Plants being sessile protect themselves from the harmful UV-B radiation by synthesizing flavonoids to screen UV-B and also by inducing antioxidant defence system. sUV-B radiation negatively affected the growth of wheat seedlings but the response varied amongst the cultivars. Leaf injury was maximum in cv. PBW154 and minimum in HD2824. Values of sensitivity index also revealed that HD2824 was least sensitive to sUV-B, while PBW154 was most sensitive. All the assessed biochemical parameters corresponded well with the sensitivity index of different cultivars of wheat.

Keywords UV-B · Wheat · Injury · Cultivar · Sensitivity

Introduction

Anthropogenic production of ozone (O_3) depleting substances has led to a reduction of stratospheric O_3 resulting

in more penetration of ultraviolet-B (UV-B) radiation on Earth's surface. While the emission of O_3 depleting substances is stabilizing, or even decreasing, substantial recovery of the O_3 layer is not expected before 2050. The amount of UV-B radiation reaching a particular place on the Earth's surface mainly depends upon its latitude as well as on the O_3 profile above it. The equatorial region is predicted to receive more UV-B radiation owing to its narrow solar angle. Plants have inherent capacity to protect themselves from the deleterious effects of UV-B with a range of repair and acclimation responses (Rozema et al. 1997; Jansen et al. 1998; Agrawal et al. 2009). Responses of plants to UV-B vary not only among the species but also among the cultivars of same species (Santos et al. 2004; Yanqun et al. 2003). Smith et al. (2000) concluded that differences in UV-B sensitivity between species represent the relative contribution of morphological, physiological and biochemical differences, but variations in sensitivity within species are usually subtle. UV-B radiation can impair all major processes of photosynthesis including photochemical reaction in thylakoid membrane, enzymatic processes in Calvin cycle, stomatal limitations to CO_2 diffusion (Allen et al. 1998).

High UV-B fluence rates directly damage photosynthetic pigments, DNA, membranes and proteins in all the organisms. UV-B radiation is considered to have the largest impact on biosphere processes, including photo degradation due to its high-energy wavelengths and capacity to penetrate stratospheric O_3 layer (Caldwell and Flint 1994; Caldwell et al. 1998). UV-B exposure is believed to enhance the amount of reactive oxygen species (ROS). Plants metabolize ROS by inducing the antioxidant defence system of low-molecular weight antioxidants such as ascorbate, glutathione and α -tocopherol and carotenoids as well as enzymes such as superoxide dismutase (SOD),

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M. Singh · S. Singh · S. B. Agrawal (✉)
Laboratory of Air Pollution and Global Climate Change,
Ecology Research Circle, Department of Botany,
Banaras Hindu University, Varanasi 221005, India
e-mail: sbagrwal56@gmail.com

catalase (CAT), peroxidase (POD) and glutathione reductase (GR) (Agrawal et al. 2009).

A substantial part of India lies in the low O₃ belt and is consequently expected to receive high flux of UV-B radiation. Sahoo et al. (2005) observed significant declining trend in total ozone column (TOC) over numerous stations lying in the northern part of India and is important in view of the Montreal Protocol that is supposed to restrict the emission of O₃-depleting substances world-wide. The Indo-Gangetic (IG) basin is characterized by significant aerosol loading (Sahoo et al. 2005). Sulphate aerosols form the major constituent of such loading (Sharma et al. 1994, 2003), comprising about 29% of total (Satheesh and Ramanathan 2000). Dust and mineral particles form a primary source of aerosol as significant aerosol build up takes over the IG basin during and preceding the summer monsoon following extensive transport of dust from the Sahara. These dust and sulphate aerosol have been shown to react with atmospheric gases (Bonasoni et al. 2001) and are likely to play an important role in the declining of TOC content in the IG basin.

The present investigation is, therefore, conducted with the objective to evaluate intraspecific differences in biochemical responses of seedlings of six wheat cultivars to ascertain their sensitivity index against supplemental UV-B radiation under natural field conditions. Screening of sensitivity would definitely help in selecting a suitable cultivar for the area receiving higher influx of UV-B radiation.

Materials and methods

Experimental site

The field experiment was conducted from February to March, 2008 at the Botanical Garden of Banaras Hindu University, Varanasi (28°18'N and 83°1'E about 76 m above mean sea level) situated in the Eastern Gangetic plains of India. Soil of the study site was sandy loam in texture (sand 45%, silt 28% and clay 27%) and has a neutral pH (7.2–7.4). During the experimental period, mean temperature ranged from 11.2 to 27.3°C, mean relative humidity from 42.3 to 77.3% and rainfall was 16.6 mm. Photosynthetically active radiation (PAR) averaged 1547 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at midday.

Plant material and treatment design

Six cultivars of wheat (*Triticum aestivum* L.) namely HUW234, HUW468, HUW 510, HD2824, PBW373, PBW154 widely grown in larger areas of Eastern Gangetic regions of India were selected as test cultivars. Wheat grains of all cultivars were sown in 36 plots of 1 × 1 m²

(six plots for each cultivar). The field was prepared by ploughing up to 20 cm depth. Recommended doses of N, P and K as urea, single superphosphate and muriate of potash (120, 60 and 40 kg ha⁻¹), respectively, were added during the preparation of the experimental field. Half dose of N and full doses of P and K were given as basal dressing and another half dose of N was given as top dressing. Wheat grains were hand sown in rows during mid February and after 1 week of germination, plants were thinned to one plant every 15 cm. Irrigation of field was done regularly to maintain the uniformity of soil moisture. Manual weeding was done two times during the entire growth period of wheat. The experimental design was split plot with treatments as main plot and cultivars as subplots. The main plots are distributed randomly in the experimental field. Each cultivar of each treatment was replicated three times.

sUV-B treatment

sUV-B was artificially provided by Q panel UV-B 313 40W fluorescent lamps (Q Panel Inc. Cleveland, OH, USA). Three lamps (120 cm long) per bank fitted 30 cm apart on a steel frame were suspended perpendicular to the planted rows of each plot. The lamps were covered by either 0.13 mm cellulose diacetate filter (transmission down to 280 nm) for supplemental UV-B (sUV-B) or 0.13 mm polyester filter (absorbed radiation below 320 nm) for the control. The control plants thus received only ambient levels of UV-B. Lamps in frames were adjusted weekly to a distance of 50 cm to provide a mean supplemental UV-B radiation of 7.6 kJ m⁻² day⁻¹ (unweighted) to plant tops for 1 h daily over the middle of photoperiod.

The UV-B irradiance at plant apices under the lamps was measured with an ultraviolet intensity meter [UVP Inc. San Gabriel, CA, USA]. The readings were converted to UVB_{BE} values by comparing with the spectro power meter (Sciencetech, Boulder, USA). Plants under polyester filtered lamps received only ambient UV-B (9.6 kJ m⁻² day⁻¹) on the summer solstice weighted against generalized plant response action spectrum of Caldwell (1971). The plants beneath cellulose diacetate film received ambient + sUV-B (ambient + 7.2 kJ m⁻² day⁻¹) that mimicked 20% reduction in stratospheric O₃ at Varanasi during clear sky condition (Green et al. 1980) normalized at 300 nm, at 0 albedo and 1.0 scatter.

Plant sampling and analysis

Plants were randomly sampled at 15 and 30 days after germination (DAG). Three replicates were taken for each measured parameters.

Growth parameters

For growth and biomass determinations, three random samples were taken by carefully digging monoliths ($10 \times 10 \times 20 \text{ cm}^3$) containing intact roots. These were

thoroughly washed by placing on a sieve of 1 mm diameter under running tap water to remove soil particles adhering to the roots. Plant height and leaf area were quantified. Leaf area was measured using portable leaf area meter (Model LI-3000, LI-COR, Inc., USA). For biomass determination,

Table 1 *F*-ratios and levels of significance of three-way ANOVA test for different parameters of wheat plants

	Age (A)	Cultivar (C)	Treatment (T)	A × C	A × T	C × T	A × C × T
Total chlorophyll	21,248.9***	16,735.2***	73,057.2***	7,433.3***	20,076.4***	2,903.2***	1,215.2***
Carotenoids	2,708.5***	13,305.9***	5,092.2***	8,262.5***	9,382.2***	15,257.9***	8,097.0***
PAL	2,561.3***	65.6***	367.7***	74.1***	25.1***	17.4***	1.41 NS
Flavonoid	481,259.7***	5,411.5***	2,896.5***	2,824.6***	2,173.1***	1,267.8***	4,761.2***
SOD	208,120.0***	1,258,045.0***	937,097.0***	142,309.8***	6,733.9***	14,236.9***	13,485.4***
APX	36.1***	36.8***	185.2***	79.3***	0.447 NS	7.33***	6.81***

PAL phenylalanine ammonia lyase, *SOD* superoxide dismutase, *APX* ascorbate peroxidase, *NS* not significant

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

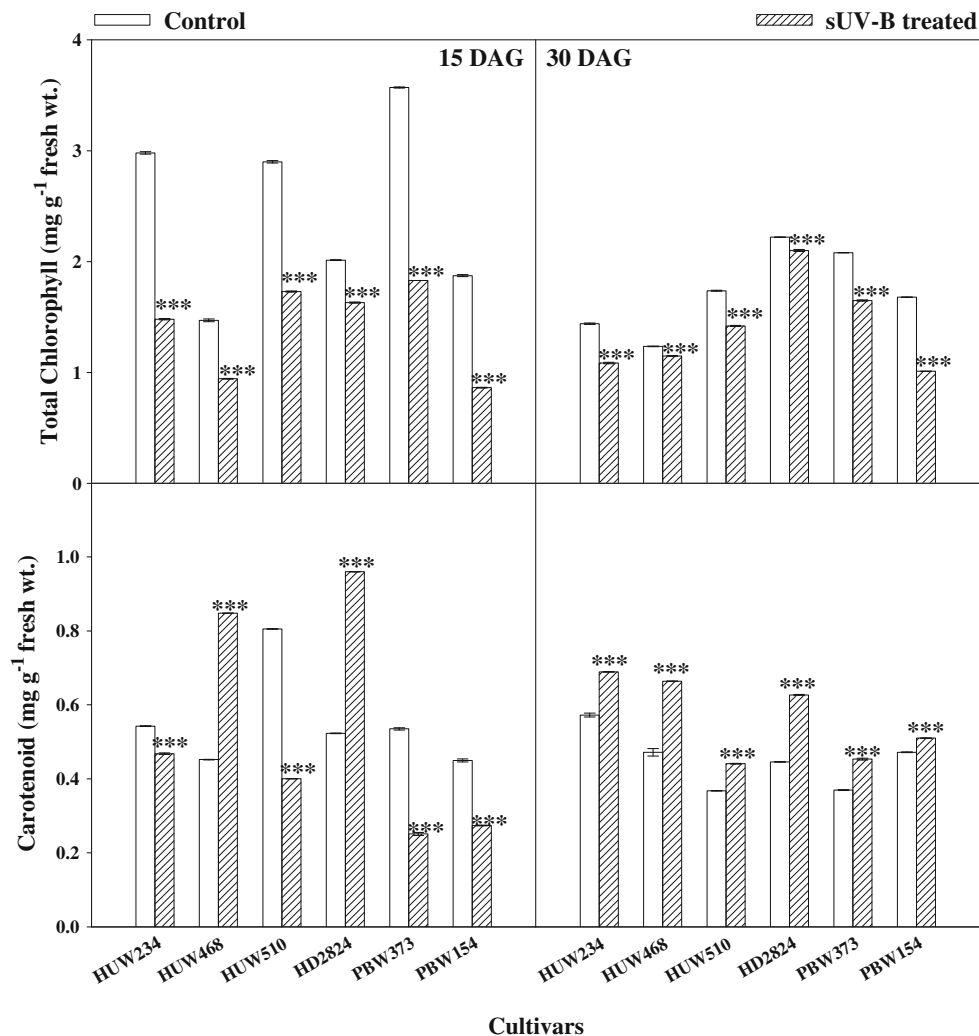


Fig. 1 Effects of sUV-B on total chlorophyll and carotenoid content of wheat plants. Values are mean \pm 1SE. Level of significance between control and sUV-B treated plants. *NS* not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

root and shoot portions were separated and oven dried at 80°C till a constant weight was achieved.

Pigments

Chlorophyll and carotenoids were extracted from the leaf disc with 80% acetone and quantified according to the methods given by Maclachlan and Zalik (1963) and Duxbury and Yentsch (1956), respectively. Flavonoids content was determined by the method described by Flint et al. (1985).

Assays of enzymes

Superoxide dismutase activity was estimated using the method of Fridovich (1974). Reaction mixture consisted of 100 mM phosphate buffer (pH 7.8), 200 mM L-methionine, 2.2 mM nitro blue tetrazolium (NBT), 0.1 mM sodium carbonate, 60 μM riboflavin and 0.1 ml enzyme extract. The ability of the extract to inhibit the photochemical reaction of NBT was determined at 650 nm on spectrophotometer (Systronics vision model 167, India). Ascorbate

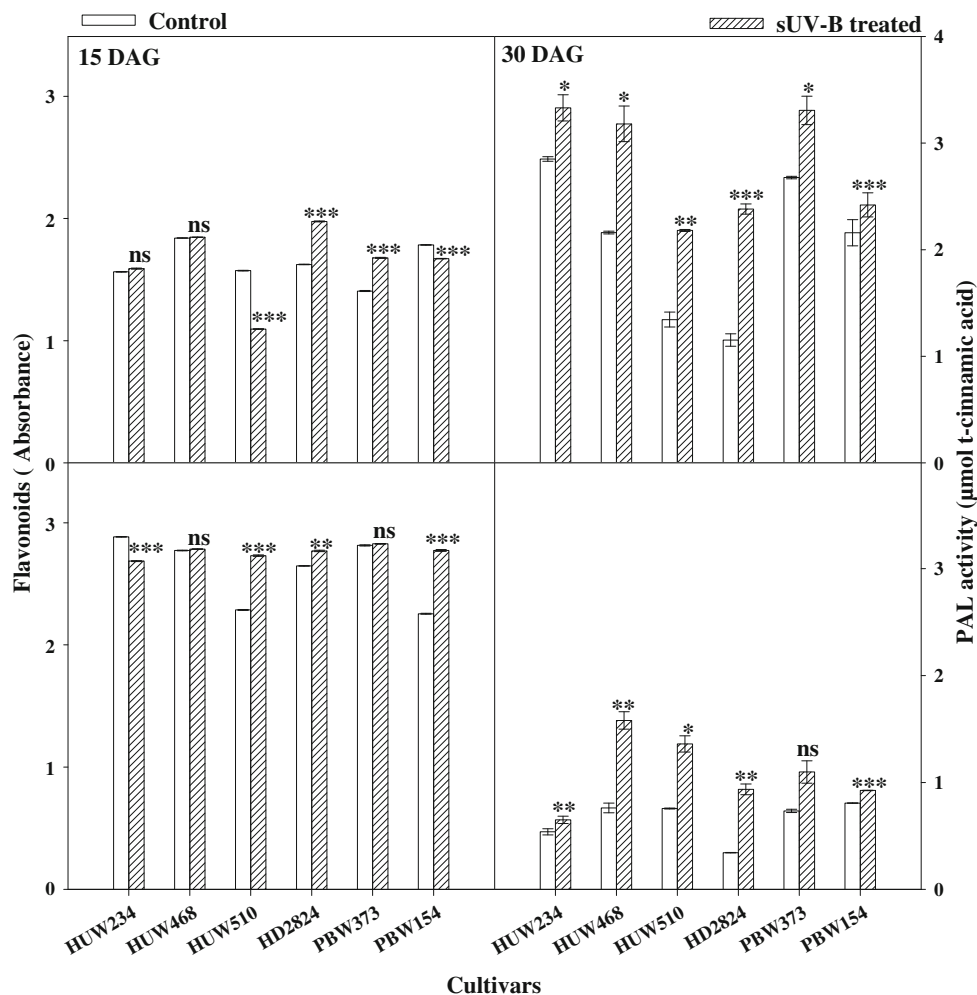
peroxidase (APX) activity was measured by the method of Nakano and Asada (1987). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.4), 0.2 mM ascorbate, 0.2 mM EDTA, 0.3 ml enzyme extract (in 50 mM sodium phosphate buffer, pH 7.4) and 0.2 ml of 2 mM H₂O₂ in a total volume of 1 ml. The decrease in absorbance was recorded at 290 nm using UV–VIS spectrophotometer. The activity was recorded at 25°C for 1 min.

For estimating phenylalanine ammonia lyase (PAL) activity, leaf tissue of 1 g was extracted in 0.2 M sodium borate buffer (pH 8.7) containing 2-mercaptoethanol and final estimation was done in the form of trans cinnamic acid using phenyl alanine at 280 nm (Rao Subba and Tower 1970). The activity of enzyme is expressed as μmole *t*-cinnamic acid ml⁻¹.

UV-B sensitivity index

A UV-B sensitivity index was determined by adding the percentage changes in biomass, plant height and leaf area [percentage change = (control-treatment)/control × 100] (Lydon et al. 1986).

Fig. 2 Effects of sUV-B on phenylalanine ammonia lyase (PAL) activity and flavonoid content of wheat plants. Values are mean ± 1SE. Level of significance between control and sUV-B treated plants. *NS* not significant, **p* < 0.05, ***p* < 0.01, ****p* < 0.001



Statistical analyses

The data of the pigments and enzymes were analyzed by three-way ANOVA test for examining the effects of age, cultivars, treatments and their interactions on various parameters. Pearson's correlation test was used to explore the correlations among changes in various parameters. Significantly different means were calculated using the 'Student's *t* test'. The entire statistical tests were performed using the SPSS software (SPSS Inc., version 14.0).

Results

Foliar symptoms

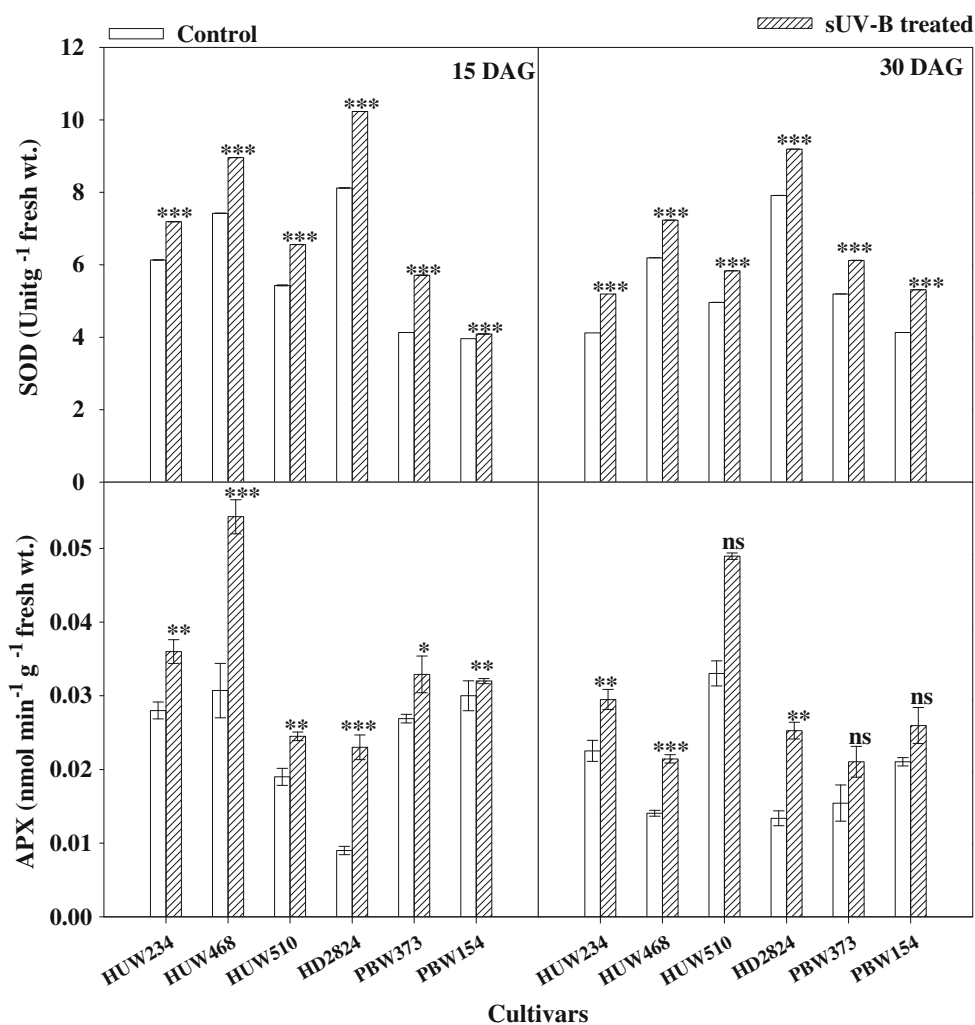
sUV-B exposure showed distinct effects in the form of visible symptoms on few mature leaves of all six cultivars of wheat. Injury was assessed initially as brown and bronze spots on the leaf surface that later resulted in chlorosis, necrosis and desiccation of the leaves. Maximum percent of

visible leaf injury was noticed in leaves of PBW 154 (61.8%) and minimum in leaves of HD2824 (47.8%), while injury was 56.3% in HUW234, 57.1% in PBW373, 47.8% in HUW468 and 56.1% in HUW510 after 20 DAG.

Photosynthetic pigments

Concentrations of chlorophyll pigments were lower in all the cultivars grown under UV-B radiation as compared to their respective controls. The magnitude of decline, however, varied between different cultivars. Three-way ANOVA test showed that variations in total chlorophyll and carotenoid concentrations were significant due to all the individual factors and their interactions (Table 1). Maximum percent reductions in chlorophyll contents were observed in PBW154 (53.9 and 39.9%) whereas minimum in HD2824 (19 and 5.4%) at 15 and 30 DAG, respectively, as compared to their controls (Fig. 1). Significant increments in carotenoids were obtained in all cultivars with maximum in HUW468 (40.7%) and HD2824 (40.6%) at 30 DAG (Fig. 1).

Fig. 3 Effects of sUV-B on superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities of wheat plants. Values are mean \pm 1SE. Level of significance between control and sUV-B treated plants. NS not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$



Flavonoids and PAL activity

Absorption profile of flavonoids showed variations in flavonoids contents under sUV-B exposure in different cultivars (Fig. 2). At wavelength 300 nm, the absorbance reached to maximal in all the cultivars at both the ages of samplings. Maximum percent increment in flavonoid content was observed in HD2824 cultivar (46.9 and 22.9%) at 15 and 30 DAG, respectively, whereas minimum increment was observed in PBW154 (Fig. 2). Results of three-way ANOVA test showed that all the individual factors as well as the interactions significantly affected the PAL activity (Table 1). Highest percent increment in PAL activity was observed in HD2824 (107 and 172%) at both the ages of observation whereas minimum PAL activity was observed in PBW154 (12.10 and 14.6%) at 15 and 30 DAG when compared to their controls (Fig. 2).

Antioxidant enzymes

APX activity increased significantly in all the cultivars of wheat under sUV-B as compared to their respective controls (Fig. 3). The significant increments in APX activities were observed in HUW234 (28.6 and 31.0%), HUW468 (76.9 and 52.4%) and HD2824 (155.6 and 89%) at 15 and 30 DAG (Fig. 3). Three-way ANOVA test revealed that APX activity varied significantly due to all the individual factors (Table 1). SOD activity increased significantly in all the cultivars at both the ages of observations (Fig. 3). Increments in SOD activities were 25.9, 16.8, 17.5, 16.1, 17.9, and 28.9% in HUW234, HUW468, HUW 510, HD2824, PBW373 and PBW154, respectively, at 30 DAG (Fig. 3). Three-way ANOVA test showed that SOD activity varied significantly due to all the individual factors and their interactions (Table 1).

UV-B sensitivity index

Relative sensitivities of the cultivars varied according to which response variable was used in the ranking based on the parameters most or least affected by UV-B in terms of biomass, plant height and leaf area (Barnes et al. 1993) (Table 2). The cultivar HD 2824 was least affected by UV-B whereas HUW234, HUW468, HUW510, PBW373 and PBW154 were the most affected. Minimum leaf area and plant height were observed in control plants of HD 2824 which did not further reduced to the level as observed in other cultivars. The calculated UV-B sensitivity index (SI) ranged from –28.9 for the least affected cv. HD2824, to –134.6 for the most affected cv. PBW154 (Table 3). Using this combined response, based on selected growth parameters, cultivars with SI greater than –20 were arbitrary classified as resistant while those with an index value less

Table 2 Effects of sUV-B on plant height, leaf area and biomass of wheat plants at 30 DAG

Cultivars	Control	sUV-B	%change
Plant height (cm plant ⁻¹)			
HUW234	27.0 ± 1.6	21.4 ± 3.4	–20.85
HUW468	25.2 ± 1.6	23.3 ± 3.5	–7.43
HUW510	27.6 ± 0.67	25.7 ± 3.4	–7.23
HD2824	22.5 ± 3.2	22.0 ± 2.7	–2.22
PBW373	28.2 ± 0.93	25.4 ± 3.2	–9.8
PBW154	31.7 ± 2.3	20.3 ± 0.88	–35.8
Leaf area (cm ² plant ⁻¹)			
HUW234	133.0 ± 35.1	78.3 ± 17.4	–41.1
HUW468	164.0 ± 65.0	114 ± 39.1	–30.5
HUW510	192.0 ± 38.8	122 ± 19.7	–36.5
HD2824	59.0 ± 11.3	53.3 ± 20.7	–9.61
PBW373	95.0 ± 22.6	66.3 ± 7.8	–30.2
PBW154	186.0 ± 44.8	96.3 ± 25.8	–48.2
Biomass (g plant ⁻¹)			
HUW234	0.443 ± 0.023	0.220 ± 0.009	–49.2
HUW468	0.307 ± 0.023	0.220 ± 0.02	–26.7
HUW510	0.323 ± 0.061	0.187 ± 0.013	–42.1
HD2824	0.450 ± 0.009	0.373 ± 0.069	–17.1
PBW373	0.270 ± 0.003	0.21 ± 0.029	–22.2
PBW154	0.83 ± 0.042	0.41 ± 0.054	–50.6

Values are mean ± 1SE

Table 3 Sensitivity index of different cultivars of wheat

Cultivars	Sensitivity index	Sensitivity
HUW234	–111.2	S
HUW468	–64.6	S
HUW154	–85.8	S
HD2824	–28.9	IS
PBW373	–62.2	S
PBW154	–134.6	MS

S sensitive, IS intermediate sensitive, MS most sensitive

than –40 were considered as sensitive. Cultivars with sensitivity indices between –20 and –40 were considered to be in class having intermediate sensitivity towards UV-B.

Discussion

The present investigation highlights the evaluation of significant differences among six widely grown cultivars of wheat against supplemental UV-B with respect to various growth and biochemical parameters. Chlorophyll contents were negatively affected by sUV-B radiation. Significant

negative correlations were observed between treatment and chlorophyll contents of five cultivars (HUW234: $r = -0.636$, $p \leq 0.05$; HUW468: $r = -0.810$, $p \leq 0.001$; HUW510: $r = -0.658$, $p \leq 0.05$; PBW373: $r = -0.715$, $p \leq 0.01$; PBW154: $r = -0.979$, $p \leq 0.001$) except HD2824 (Tables 4 and 5). So, it may be used as response indicator for assessing the degree of UV-B sensitivity in wheat cultivars. UV-B significantly decreased the chlorophyll contents in pea leaves primarily because it destroyed the structure of chloroplasts, inhibited synthesis of new chlorophyll and also increased the degradation of chlorophyll (Strid and Porra 1992). Significant positive correlations between treatment and carotenoids were observed for HUW468 ($r = 0.914$, $p \leq 0.001$) (Table 4). The carotenoids are implicated in photoprotection of both the photosystems involved in photosynthesis. Carotenoids help in protecting the photosystems due to their ability as efficient quenchers of high-energy shortwave radiation. Significant negative correlations between carotenoids and chlorophyll contents for HUW468 and HD2824 showed

that relatively low reduction in chlorophyll was because of higher content of carotenoids. Synthesis of UV-B screening compounds such as flavonoids have been observed in UV-B treated *Arabidopsis thaliana* (L.) Heynh seedlings (Ravindran et al. 2001). The accumulation of flavonoids is also considered as a defence mechanism against UV-B as they protect the mesophyll tissue being photosynthetic machinery through epidermal screening of the UV-B radiation. In addition, it has been shown that some of these compounds may have antioxidant properties (Rice-Evans et al. 1997). Accumulation of flavonoids under UV irradiation has been reported (Mazza et al. 1999; Agrawal and Rathore 2007; Kumari et al. 2010). In the present experiment, inspite of an increase of protective compounds, damage to the chloroplasts are observed as indicated by the decrease in chlorophyll content. Maximum increase in flavonoid concentration was recorded for cv. PBW154. Significant positive correlation was found between treatment and PAL activity for HUW510 ($r = 0.703$, $p \leq 0.05$) and HD2824 ($r = 0.612$, $p \leq 0.05$) (Tables 4 and 5). Even

Table 4 Correlation coefficient (r) between various measured parameters under different age and treatment in wheat cvs. HUW234, HUW468 and HUW510 at 30 DAG

	Total chlorophyll	Carotenoid	PAL	Flavonoid	SOD	APX
HUW234						
Age	-0.664*	0.784**	-0.988***	0.993***	-0.883***	-0.544 NS
Treatment	-0.636*	0.144 NS	0.117 NS	-0.071 NS	0.469 NS	0.741**
Total chlorophyll		-0.376 NS	0.553 NS	-0.651*	0.289 NS	-0.121 NS
Carotenoid			-0.800**	0.731**	-0.624*	-0.336 NS
PAL				-0.983***	0.927***	0.652*
Flavonoid					-0.911***	-0.590*
SOD						0.829***
HUW468						
Age	-0.039 NS	-0.256 NS	-0.841***	0.998***	-0.748***	-0.796***
Treatment	-0.810***	0.914***	0.516 NS	0.010 NS	0.652*	0.506 NS
Total chlorophyll		-0.913***	-0.415 NS	0.045 NS	-0.572 NS	0.525 NS
Carotenoid			-0.706*	-0.248 NS	0.827***	0.750**
PAL				0.837***	0.973***	0.964***
Flavonoid					-0.742**	-0.792**
SOD						0.959***
HUW510						
Age	-0.652*	-0.562 NS	-0.687*	0.931***	-0.511 NS	0.848***
Treatment	-0.658*	-0.472 NS	0.703*	-0.013 NS	0.852***	0.461 NS
Total chlorophyll		0.933***	-0.055 NS	-0.460 NS	-0.269 NS	-0.768**
Carotenoid			-0.022 NS	-0.269 NS	-0.190 NS	-0.539 NS
PAL				-0.690*	0.965***	0.274 NS
Flavonoid					-0.527 NS	0.866***
SOD						-0.065 NS

NS not significant

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 5 Correlation coefficient (*r*) between various measured parameters under different age and treatment in wheat cvs. HD2824, PBW373 and PBW154 at 30 DAG

	Total chlorophyll	Carotenoid	PAL	Flavonoid	SOD	APX
HD2824						
Age	0.765**	-0.523 NS	-0.757**	0.960***	-0.337 NS	0.261 NS
Treatment	-0.569 NS	0.780**	0.612*	0.251 NS	0.914***	0.933***
Total chlorophyll		-0.946***	-0.989***	0.556 NS	-0.845***	-0.344 NS
Carotenoid			0.947***	-0.265 NS	0.970***	0.621*
PAL				-0.547 NS	0.863***	0.494 NS
Flavonoid					-0.067 NS	0.494 NS
SOD						0.781**
PBW373						
Age	-0.550 NS	0.086 NS	-0.964***	0.989***	0.493 NS	-0.808***
Treatment	-0.715**	-0.476 NS	0.231 NS	0.11 NS	0.842***	0.416 NS
Total chlorophyll		0.670*	0.339 NS	-0.666*	-0.968***	0.144 NS
Carotenoid			-0.245 NS	-0.055 NS	-0.549 NS	-0.269 NS
PAL				-0.922***	-0.267 NS	0.915***
Flavonoid					0.607*	-0.751**
SOD						-0.044 NS
PBW154						
Age	-0.027 NS	0.715**	-0.977***	0.903***	0.638*	-0.764**
Treatment	-0.979***	-0.378 NS	0.130 NS	0.232 NS	0.601*	0.377 NS
Total chlorophyll		0.469 NS	-0.109 NS	-0.180 NS	-0.510 NS	-0.311 NS
Carotenoid			-0.774**	0.770**	0.512 NS	-0.586*
PAL				-0.864***	-0.568 NS	0.830***
Flavonoid					0.890***	-0.540 NS
SOD						-0.182 NS

NS not significant

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

with increments in PAL activities, flavonoid contents reduced in HUW234 ($r = -0.800$, $p \leq 0.01$), HUW468 ($r = -0.706$, $p \leq 0.01$), PBW373 ($r = -0.922$, $p \leq 0.001$) and PBW154 ($r = -0.864$, $p \leq 0.001$). Results of present study also showed that UV-B triggered the PAL activity vis-à-vis flavonoid biosynthesis. PAL activity increased significantly under UV-B exposure of wheat cultivars at both the ages of observations.

Results of the present experiment performed under natural field conditions clearly showed varietal differences in cellular antioxidant defence system of wheat to combat the oxidative stress induced by UV-B. SOD activities correlated significantly with all the cultivars except HUW234 (HUW468: $r = 0.652$, $p \leq 0.05$; HUW510: $r = 0.852$, $p \leq 0.001$; HD2824: $r = 0.914$, $p \leq 0.001$; PBW373: $r = 0.842$, $p \leq 0.001$; PBW154: $r = 0.601$, $p \leq 0.05$) (Tables 4, 5). Our findings showed that SOD activity increased in all the test cultivars. Since, SOD controls the concentration of O_2^- and its derivatives (H_2O_2 and OH), it is defined as a key enzyme of antioxidant

defence system (Bowler et al. 1992). In a field study, supplemental UV-B increased SOD activity in wheat and bean (Agrawal and Rathore 2007), and caused different responses among soybean cultivars (Yanqun et al. 2003). In addition to SOD, APX activity was enhanced simultaneously by UV-B. This was in consistent with the results of several other studies conducted in controlled chambers (Yannarelli et al. 2006; Takeuchi et al. 1995; Rao et al. 1996; Landry et al. 1995) or in the field (Mazza et al. 1999) and suggested that APX played an important role in the control of endogenous H_2O_2 content. Significant positive correlations between treatment and APX activity were observed in cvs. HUW234 ($r = 0.741$, $p \leq 0.01$) (Table 4) and HD2824 ($r = 0.933$, $p \leq 0.001$) (Table 5). Enhanced SOD activities also stimulated the increments in APX activities of cvs. HUW234, HUW468 and HD2824 (Tables 4, 5). Therefore, although production of ROS were noticed due to UV-B stress, increased stimulation of antioxidant defence system possibly alleviated the UV-B induced damage by quenching toxic radicals.

Conclusions

All six wheat cultivars showed significant differences in their response to UV-B radiation. Cultivar HD2824 showed relatively less sensitivity to UV-B than rest of the cultivars whereas PBW154 showed maximum sensitivity against all the growth parameters including total biomass. The percent reduction in photosynthetic pigments was minimum in HD2824 along with maximum induction of UV-B absorbing pigments, flavonoids. Significant positive correlation between treatment and carotenoid content was only observed in cv. HUW468. Increment in SOD activity was minimum in HD2824 and maximum in PBW154 whereas APX activity increased maximally in HD2824 and minimally in PBW154. Enhanced SOD activity stimulated the activity of APX in cvs. HUW234, HUW468 and HD2824. The biochemical responses of different cultivars were in agreement with their sensitivity index.

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References

- Agrawal SB, Rathore D (2007) Changes in oxidative stress defense in wheat (*Triticum aestivum* L.) and mung bean (*Vigna radiata* L.) cultivars grown with or without mineral nutrients and irradiated by supplemental ultraviolet-B. *Environ Exp Bot* 59:21–23
- Agrawal SB, Singh S, Agrawal M (2009) Ultraviolet-B induced changes in gene expression and antioxidants in plants. *Adv Bot Res* 52:47–86
- Allen DJ, Nogue S, Baker NR (1998) Ozone depletion and increased UV-B radiation: is there a real threat to photosynthesis? *J Exp Bot* 49:1775–1788
- Barnes PW, Maggard S, Holman SR, Vergara BS (1993) Intraspecific variation in sensitivity to UV-B radiation in rice. *Crop Sci* 33:1041–1046
- Bonasoni P, Calzolari F, Cristofanelli P, Bonafe U, Evangelisti F, Vandingenen R, Balkanski Y (2001) Ozone and aerosol correlation during Sahara dust transport episodes at Mount Climone during MINATROC project. In: 8th European symposium on the physico-chemical behavior of atmospheric pollutants, Torino, pp 17–20
- Bowler C, Van Montagu M, Inze D (1992) Superoxide dismutase and stress tolerance. *Ann Rev Plant Physiol Plant Mol Biol* 43(1):83–116
- Caldwell MM (1971) Solar ultraviolet radiation and the growth and development of higher plants. In: Giese AC (ed) *Photophysiology*. Academic Press, New York, pp 131–171
- Caldwell MM, Flint SD (1994) Stratospheric ozone reduction, solar UV-B radiation, and terrestrial ecosystems. *Clim Change* 28:375–394
- Caldwell MM, Bjorn LO, Bornman JF, Flint SD, Kulandaivelu G, Teramura AH, Tevini M (1998) Effects of solar ultraviolet radiation on terrestrial ecosystems. *J Photochem Photobiol B Biol* 46:40–52
- Duxbury AC, Yentsch CS (1956) Plankton pigment monographs. *J Mar Res* 15:19–101
- Flint SD, Jordan WP, Caldwell MM (1985) Plant protective response to enhanced UV-B radiation under field conditions: leaf optical properties and photosynthesis. *Photochem Photobiol* 41:95–99
- Fridovich I (1974) Superoxide dismutase. *Adv Enzymol* 41:35–97
- Green AES, Cross KR, Smith LA (1980) Improved analytical characterization of ultraviolet skylight. *Photochem Photobiol* 31:59–65
- Jansen MAK, Gaba V, Greenberg BM (1998) Higher plants and UV-B radiation: balancing, damage, repair and acclimation. *Trends Plant Sci* 3:131–135
- Kumari R, Singh S, Agrawal SB (2010) Responses of ultraviolet-B induced antioxidant defense system in a medicinal plant *Acorus calamus*. *J Environ Biol* 31(6):907–911
- Landry LG, Chapple CCS, Last RL (1995) Arabidopsis mutants lacking phenolic sunscreens exhibit enhanced UV-B injury and oxidative damage. *Plant Physiol* 109:1159–1166
- Lydon J, Teramura AH, Summers EG (1986) Effects of ultraviolet-B radiation on growth and productivity of field grown soybean. In: Worrest RC (ed) *Stratospheric ozone reduction, solar ultraviolet radiation and plant life*. Springer, Berlin, pp 313–325
- Maclachlan S, Zalik S (1963) Plastid structure, chlorophyll concentration and free amino acid composition of a chlorophyll mutant of barley. *Can J Bot* 41:1053–1062
- Mazza CA, Battista D, Zima AM, Szwarcberg-Bracchitta M, Giordano CV, Acevedo A, Scopel AL, Ballare CL (1999) The effects of solar ultraviolet-B radiation on the growth and yield of barley are accompanied by increased DNA damage and antioxidant responses. *Plant Cell Environ* 22:61–70
- Nakano Y, Asada K (1987) Purification of ascorbate peroxidase in spinach chloroplast, its inactivation in ascorbate depleted medium and reactivation by monodehydroascorbate radical. *Plant Cell Physiol* 28:131–140
- Rao Subba PV, Tower GHN (1970) L-phenylalanine ammonia-lyase (*Ustilago hordei*). In: Colowick SP, Kaplan NO (eds) *Methods in enzymology*. Academic Press, New York, p 581
- Rao MV, Paliyath G, Ormrod DP (1996) Ultraviolet-B and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiol* 110:125–136
- Ravindran KC, Mahes Kumar N, Amirthalingam V, Ranganathan R, Chellappan KP, Kulandaivelu G (2001) Influence of UV-B supplemental radiation on growth and pigment content in *Suaeda maritima* L. *Biol Plant* 44:467–469
- Rice-Evans CA, Miller NJ, Paganga G (1997) Antioxidant properties of phenolic compounds. *Trends Plant Sci* 2:152–159
- Rozema J, Van de Staaij J, Björn LO, Caldwell MM (1997) UV-B as an environmental factor in plant life: stress and regulation. *Trends Ecol Evol* 12:22–28
- Sahoo A, Sarkar S, Singh RP, Kafatos M, Summers ME (2005) Declining trend of total ozone column over the northern parts of India. *Int J Rem Sens* 26(16):3433–3440
- Santos I, Fernanda F, Jose MA, Roberta S (2004) Biochemical and ultrastructural changes in leaves of potato plants grown under supplementary UV-B radiation. *Plant Sci* 167:925–929
- Satheesh SK, Ramanathan V (2000) Large differences in tropical aerosol forcing at the top of the atmosphere and Earth's surface. *Nature* 405:60–63
- Sharma M, Mcbean EA, Ghosh U (1994) Prediction of atmospheric sulphate deposition at sensitive receptors in northern India. *Atmos Environ* 29:2157–2162
- Sharma M, Kiran YNVM, Shandilya KK (2003) Investigations into formation of atmospheric sulfate under high PM10 concentrations. *Atmos Environ* 37:2005–2013

- Smith JL, Buritt DJ, Bannister P (2000) Shoot dry weight, chlorophyll and UV-B absorbing compounds as indicators of a plant's sensitivity to UV-B radiation. *Ann Bot* 86:1057–1063
- Strid Å, Porra RJ (1992) Alterations in pigment content in leaves of *Pisum sativum* after exposure to supplementary UV-B. *Plant Cell Physiol* 33:1015–1023
- Takeuchi Y, Fukeimoto R, Hirokazu K, Sakaki T, Kitao M (1995) Peroxidation of lipids and growth inhibition induced by UV-B radiation. *Plant Cell Rep* 14(9):566–570
- Yannarelli GG, Gallego SM, Tomaro ML (2006) Effect of UV-B radiation on the activity and isoforms of enzymes with peroxidase activity in sunflower cotyledons. *Environ Exp Bot* 56:174–181
- Yanqun Z, Yuan L, Haryan CJ, Ianjun C (2003) Intraspecific differences in physiological response to 20 soybean cultivars to enhanced ultraviolet-B radiation under field conditions. *Environ Exp Bot* 50:87–97