Photosynth Res (2013) 115:123–138
DOI 10.1007/s11120-013-9841-2

REGULAR PAPER

Differential sensitivity of spinach and amaranthus to enhanced UV-B at varying soil nutrient levels: association with gas exchange, UV-B-absorbing compounds and membrane damage

Suruchi Singh · Madhoolika Agrawal · S. B. Agrawal

Received: 29 May 2012 / Accepted: 26 April 2013 / Published online: 18 May 2013

Abstract The metabolic reasons associated with differential sensitivity of C_3 and C_4 plant species to enhanced UV-B under varying soil nutrient levels are not well understood. In the present study, spinach (*Spinacia oleracea* L. var All Green), a C_3 and amaranthus (*Amaranthus tricolor* L. var Pusa Badi Chaulai), a C_4 plant were subjected to enhanced UV-B (280–315 nm; 7.2 kJ m^{-2} day^{-1}) over ambient under varying soil nutrient levels. The nutrient amendments were recommended Nitrogen (N), Phosphorus (P), Potassium (K), 1.5× recommended NPK, 1.5× recommended N and 1.5× recommended K. Enhanced UV-B negatively affected both the species at all nutrient levels, but the reductions varied with nutrient concentration and combinations. Reductions in photosynthetic rate, stomatal conductance and chlorophyll content were significantly more in spinach compared with amaranthus. The reduction in photosynthetic rate was maximum at 1.5× recommended K and minimum in 1.5× NPK amended plants. The oxidative damage to membranes measured in terms of malondialdehyde content was significantly higher in spinach compared with amaranthus. Enhanced UV-B reduced SOD activity in both the plants except in amaranthus at 1.5× recommended K. POX activity increased under enhanced UV-B at all nutrient levels in amaranthus, but only at 1.5× K in spinach. Amaranthus had significantly higher UV-B-absorbing compounds than spinach even under UV-B stress. Lowest reductions in yield and total biomass under enhanced UV-B compared with ambient were observed in amaranthus grown at 1.5× recommended NPK. Enhanced UV-B did not significantly change the nitrogen use efficiency in amaranthus at all NPK levels, but reduced in spinach except at 1.5× K. These findings suggest that the differential sensitivity of the test species under enhanced UV-B at varying nutrient levels is due to varying antioxidative and UV-B screening capacity, and their ability to utilize nutrients. Amaranthus tolerated enhanced UV-B stress more than spinach at all nutrient levels and 1.5× recommended NPK lowered the sensitivity maximally to enhanced UV-B with respect to photosynthesis, biomass and yield. PCA score has also confirmed the lower sensitivity of amaranthus compared with spinach with respect to the measured physiological and biochemical parameters.

Keywords UV-B · Spinach · Amaranthus · Oxidative damage · Nitrogen use efficiency

S. Singh · M. Agrawal (✉) · S. B. Agrawal
Laboratory of Air Pollution and Global Climate Change, Department of Botany, Banaras Hindu University, Varanasi 221005, India
e-mail: madhoo58@yahoo.com

Introduction

Since higher plants are immobile, they are inevitably exposed to solar UV-B radiation. Natural variations in UV-B irradiances at the ground are produced due to natural latitudinal gradient in total atmospheric ozone column thickness, prevailing solar angles, elevation above sea level and optical amplification effect (Cabrera et al. 1995). The amount of UV-B radiation reaching tropical latitudes is higher than in temperate because the lower solar zenith angle leads to a less atmospheric UV-B absorption in tropics. Numerous stations lying in the northern India showed significant declining trend in total ozone column (TOC), suggesting the potential vulnerability of plants to increased UV-B under field conditions (Sahoo et al. 2005).

Some plants are sensitive to even ambient fluences (Krizek et al. 1997), while many plants appear quite

tolerant to even high fluences of UV-B (Allen et al. 1998). UV-B photons cause cellular damage by generating DNA photoproducts and through direct damage to proteins, lipids and RNA (Casati and Walbot 2003). Enhanced UV-B exposure to plants can impair all major photosynthesis processes including photochemical reaction in thylakoid membrane, enzymatic processes in the Calvin cycle and stomatal limitation to CO_2 (Allen et al. 1998). Cell membranes are regarded as one of the potential targets to UV-B (Björn 1996). Studies have shown that enhanced UV-B irradiation decreased plasma membrane ATPase (Murphy 1983), increased membrane permeability (Imbrie and Murphy 1982), caused damage to unsaturated fatty acids of membrane lipids (Predieri et al. 1995) and even changed the properties of thylakoid membrane (Murphy 1983).

Previous studies have shown that the plant's response to UV-B can be modified by other environmental factors like photosynthetic photon flux density (PPFD) (Adamse and Britz 1992), atmospheric CO_2 concentration (Visser et al. 1997), water availability (Balakumar et al. 1993) and nutrient supply (Singh et al. 2010). Studies on the combined effects of UV-B and other environmental factors on plant species are of importance for the accurate assessment of potential consequences of stratospheric ozone layer reduction. C_4 plants are reported to be better adapted to water stress than C_3 plants (Uzilday et al. 2012). C_3 and C_4 plant species have evolved in different climates and consequently differ from each other both structurally and functionally, and for their climatic requirements as well (Ward et al. 1999; Nayyar 2003). Such differences may allow different defence strategies including antioxidative potential, UV-B screening capacity and nutrient use efficiency among the plant species. Hence, the interactive effects of enhanced UV-B and nutrients were assessed on spinach (*Spinacia oleraceae* L. cv All green), a C_3 and amaranthus (*Amaranthus tricolor* L. cv Pusa Badi Chaulai), a C_4 plant species under natural field conditions.

The main objectives of the study were to compare the responses of both the species at ambient UV-B (5.8 kJ m^{-2} day^{-1} biologically effective UV-B) and enhanced UV-B over ambient (7.2 kJ m^{-2} day^{-1} biologically effective UV-B above ambient; Caldwell 1971) with respect to (i) photosynthesis, stomatal conductance, water use efficiency (WUE), chlorophyll fluorescence, photosynthetic pigments, UV-B-absorbing compounds, lipid peroxidation (LPO), antioxidative enzymes, metabolites, total biomass, root shoot ratio (RSR) and yield, (ii) nitrogen use efficiency (NUE) and (iii) the modification in above responses at different doses of nutrients and their combinations. We hypothesize that C_3 and C_4 plant species will respond differently under enhanced UV-B at varying nutrient levels due to physiological and metabolic differences. The combination of 1.5× NPK will help the plant species more efficiently under enhanced UV-B by adapting different defence strategies compared with other combinations. The plant species which will have better NUE will show higher tolerance to enhanced UV-B.

Materials and methods

Experimental site and plant material

The field experiment was conducted from March 2010 to April 2010 at the Botanical Garden of the Banaras Hindu University, Varanasi, Uttar Pradesh (25°81′N, 83°1′E and 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 had a pH close to neutral (7.2–7.4). Variations in climatological data during the study period are shown in Fig. 1. Mean, minimum and maximum temperatures and PPFD were higher during April compared with March, while relative humidity showed a contrasting trend (Fig. 1).

The test plant species were spinach (*S. oleraceae* L. cv All green) and amaranthus (*A. tricolor* L. cv Pusa Badi Chaulai). Both the varieties are high-yielding varieties developed by Indian Agricultural Research Institute (IARI), New Delhi and are widely grown in northern Indian conditions.

Experimental design and nutrient application

The experimental design was a split plot with a UV-B treatment as main plot and nutrient treatments and plant species as subplots randomized within the whole plots. Each treatment had three replicate plots. In total there were 48 plots of 1 × 1 m^2 each. The experiment had two factors: (i) UV-B treatment, and (ii) N, P and K amendment. The four NPK amendments were recommended dose of NPK (F_0), 1.5× recommended dose of NPK (F_1), 1.5× recommended dose of N (F_2) and 1.5× recommended dose of K (F_3). Recommended dose of NPK is the optimum nutrient required by the plants and 1.5× NPK was chosen to test the plant's response to UV-B at higher than required nutrient level. Higher than recommended dose of N is used to test the plant's response to single nutrient under enhanced UV-B, and 1.5× K was used to test the role of K in membrane damage protection under enhanced UV-B.

For convenience, control plants grown at ambient level of UV-B were designated as F_0C, F_1C, F_2C and F_3C with corresponding UV-B treated plants as F_0T, F_1T, F_2T and F_3T, respectively. Recommended dose of NPK for spinach and amaranthus is declared by IARI. The recommended dose of NPK was 80:40:40 kg ha^{-1} for spinach and 75:50:40 kg ha^{-1}, respectively, for amaranthus. N, P and K were given in the form of urea, single super phosphate and

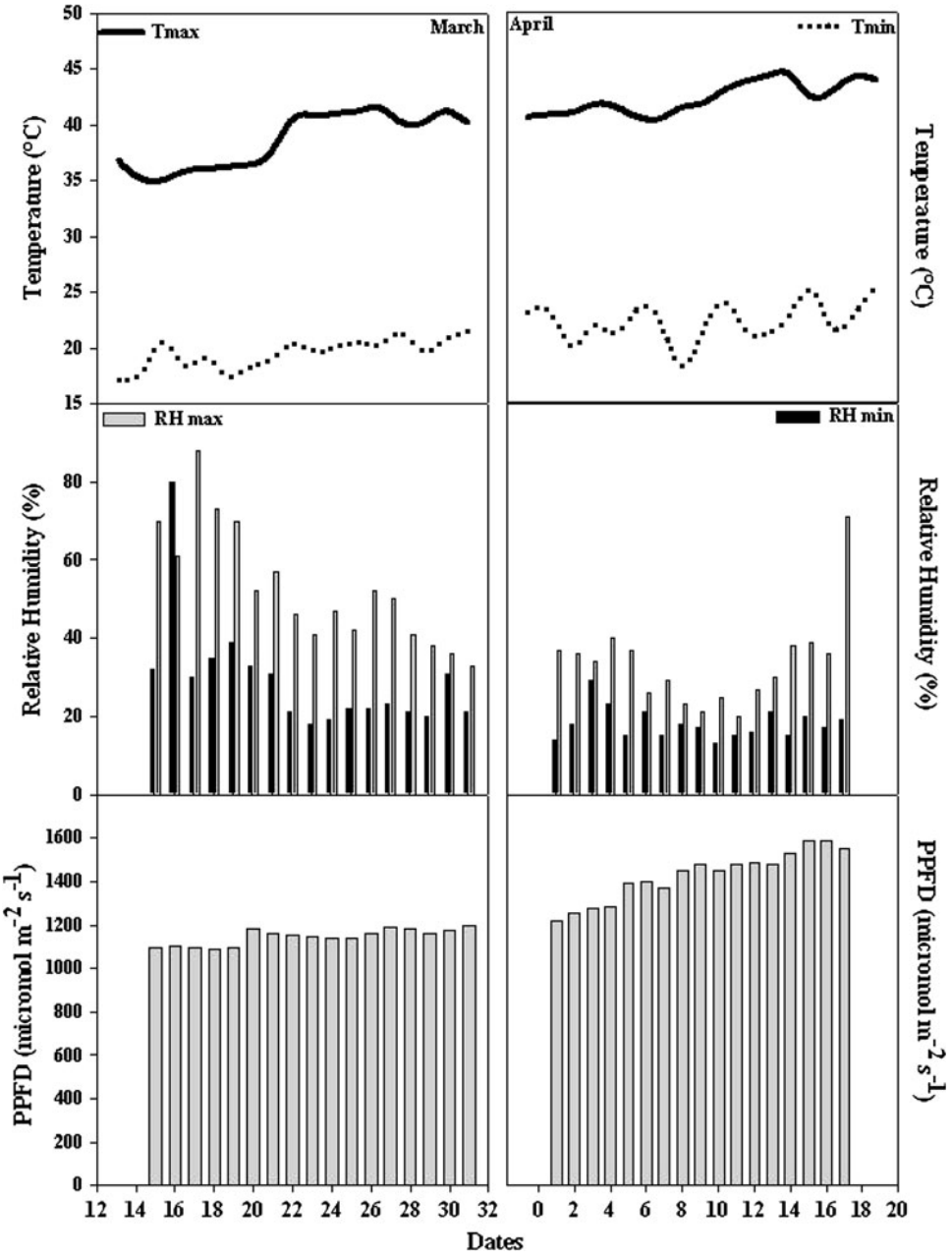

Fig. 1 Variations in the climatological conditions during the study period

muriate of potash, respectively. A 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 after 7 days after germination (DAG).

Genetically uniform seeds of spinach and amaranthus procured from Indian Institute of Vegetable Research, Varanasi were sown in rows (30 cm apart) in each plot. There were three rows in each plot. There were 18 plants in each plot with 6 plants in each row. After germination, plants were thinned to one plant every 15 cm for uniformity in growth. Plants were watered every alternate day with same amount of water through channel irrigation.

Enhanced UV-B treatment

Enhanced UV-B was artificially provided by Q-panel UV-B 313 40 W fluorescent lamps (Q panel Inc. Cleveland, OH, USA). For each treatment, three replicate plots were maintained with a separate frame. Each steel frame contained three lamps (120 cm long) fitted 30 cm apart and were suspended perpendicular to the planted rows (3) of each plot. The lamps were covered by either 0.13 mm cellulose diacetate filter (transmission down to 280 nm) for enhanced UV-B radiation or 0.13 mm polyester filter (absorbed radiation <320 nm) for the control.

Ultraviolet irradiance was measured with a double monochromator spectroradiometer (Scientech, Boulder, CO). Plants under polyester filter lamps received only ambient UV-B (5.8 kJ m^{-2} day^{-1} biologically effective UV-B) on the summer solstice weighted against generalized plant response action spectrum of Caldwell (1971). The plants beneath cellulose diacetate film received ambient + enhanced UV-B (7.2 kJ m^{-2} day^{-1} biologically effective UV-B; Caldwell 1971) that mimicked 20 % reduction in stratospheric ozone at Varanasi during clear sky condition (Green et al. 1980) normalized at 300 nm, at 0 albedo and 1.0 scatter. Lamps in frames were adjusted weekly to a distance of 45 cm above plant canopy to provide a mean enhanced UV-B of 7.2 kJ m^{-2} day^{-1} (weighted; Caldwell 1971) for 3 h daily over the middle of photoperiod (10.00 am to 1.00 pm).

Gas exchange and chlorophyll fluorescence

Photosynthetic rate (Ps) and stomatal conductance (gs) were measured using portable photosynthetic system (Model LI-6200, LI-COR, USA) at 20 days after germination (DAG). The measurements were made on the third fully expanded leaf from the top of the plant species on cloud free days between 08.00 and 10.00 h local time on three randomly selected plants in each plot. During the measurements, PPFD ranged between 1100 and 1500 µmol m^{-2} s^{-1}. The system was calibrated using a known CO_2 source of 509 ppm concentration. During measurements of photosynthesis, the mean temperature ranged between 29.8 and 31.8 °C, relative humidity ranged between 37.3 and 38.1 %. Intrinsic WUE was calculated as a ratio of photosynthesis to stomatal conductance.

Chlorophyll fluorescence characteristics such as initial fluorescence (F_0), maximum fluorescence (Fm), variable fluorescence ($Fv = Fm - F_0$) and Fv/Fm ratio were measured between 8.00 and 10.00 h using portable plant efficiency analyzer (PEA, Hansatech Instruments Ltd., UK) on the same leaves where Ps was measured. Leaf clips for dark adaptation were placed on the adaxial side of the leaves for 20 min before measurement at saturating flash of 3000 µmol m^{-2} s^{-1}.

Pigments, antioxidants and metabolites

Random samplings of plants were performed in triplicate at 20 DAG from each plot and leaves were cut and stored at −20 °C before analyses of photosynthetic pigments, antioxidants and metabolites.

Pigments

For pigment determination, 500 mg of leaf samples were homogenized in 20 ml of 80 % acetone and centrifuged at 6000× rpm for 15 min. The optical densities (O.D.) of supernatant were measured at 480 and 510 nm wavelengths for carotenoids and 645 and 663 nm for chlorophylls on a UV–Vis spectrophotometer (Systronics Model 119, India). The amounts of chlorophyll a and b and carotenoids were calculated by using the formulae given by Maclachlan and Zalik (1963) and Duxbury and Yentsch (1956), respectively. For determination of UV-B-absorbing compounds, 0.1 g of leaf discs of 100 mm^2 were taken and extracted in 10 ml of acidified methanol (79:30:1 v/v, methanol, water, HCl) according to the procedure of Mirecki and Teramura (1984). Extract absorbance at 305 nm wavelength was measured on a UV–Vis spectrophotometer (Model 119, Systronics, India), as a measure of UV-B-absorbing compounds.

Lipid peroxidation

Lipid peroxidation in the leaf tissues was determined in terms of malondialdehyde (MDA, a product of LPO) content by trichloroacetic acid (TCA) reaction as described by Heath and Packer (1968).

Antioxidants and metabolites

Peroxidase (POX) activity was determined by using the method of Britton and Mehley (1955). Superoxide dismutase (SOD) activity was assayed according to the method of Fridovich (1974). For protein extraction, fresh leaves were homogenized in tris buffer (0.1 M) followed by mixing of TCA (10 %) and then dissolved into 0.1 N NaOH. Estimation of protein was performed by the method of Lowry et al. (1951). For ascorbic acid, leaf samples were homogenized in oxalic acid and NaEDTA extraction solution. 2,6-dichlorophenol-indophenol dye was used to develop colour and the absorbance was taken at 520 nm. Ascorbic acid content was quantified using the method of Keller and Schwager (1977). Phenol content was estimated by homogenizing the leaf sample in acetone and then using Folin–Ciocalteu reagent and Na_2CO_3 (Bray and Thorpe 1954). From the standard curve prepared from catechol, different concentrations of phenols in test samples were calculated in mg g^{-1}.

Total biomass and yield

For biomass estimation, root and shoot portions of plants were oven dried separately at 80 °C till constant weight was achieved. Dry weights of component samples were measured separately. Fresh weights of leaves (consumed portion) of five different plants of each plot were taken for yield estimation of amaranthus and spinach plants. RSR was calculated from biomass data using formula given by Hunt (1982).

Nitrogen and nitrogen use efficiency

For nitrogen analysis, oven-dried samples of leaves were ground in a stainless steel grinder and passed through 2-mm sieve. Total N was quantified using the micro-Kjeldahl technique in a Gerhardt Automatic N Analyzer (Automatic Analyzer, Model KB8S, Bonn, Germany). NUE was determined as dry matter accumulation production per unit nitrogen content (Moll et al. 1982) at the level of edible part, i.e. leaves.

Statistical analyses

The statistical significance of the data for biochemical and physiological parameters were tested through two way analysis of variance (ANOVA) test to examine the individual and combined effects of nutrient (N) and treatment (T). Significantly, different means between control and its respective enhanced UV-B treated plants were calculated using the 'Students *t* test'. All the statistical tests were performed using SPSS software (SPSS Inc., version 14.0). The entire data set of 48 plots was subjected to a principal component analysis (PCA) using the Varimax method. This analysis allows the identification of interrelated variables.

Results

The results of two-way ANOVA showed that all the measured parameters in amaranthus were significantly affected by nutrients, treatment except NUE, initial fluorescence and Fm, and their interactions except NUE, POX and biomass (Table 1). In spinach, all the parameters except biomass under N × T interaction varied significantly with individual factors and their interactions (Table 1). Photosynthesis reduced significantly in both the plant species under enhanced UV-B with minimum in F_1T treatment (amaranthus; 18.4 % and spinach; 30.9 %) and maximum in F_0T (37.1 %) amaranthus and F_2T (41.8 %) spinach (Fig. 2). Stomatal conductance declined significantly under enhanced UV-B with maximum decline in F_0T (53.4 %) and minimum in F_1T (5.7 %) amaranthus, whereas spinach exhibited maximum decline in F_1T (81.6 %) and minimum in F_3T (40.5 %) (Fig. 2). Intrinsic WUE increased in F_1C amaranthus (4.04-folds), and F_0T (1.47-folds) and F_1T (3.77-folds) spinach under enhanced UV-B compared with their controls (Fig. 2).

Under enhanced UV-B, initial fluorescence increased significantly in F_0T (6.6 %), whereas decreased in F_1T (4.9 %) amaranthus; however, in spinach, it increased in F_0T (6.2 %), F_1T (3.4 %), F_2T (8.6 %) and F_3T (17.3 %) (Table 2). Fm increased in F_0T (11.9 %) and F_3T (1.2 %), whereas decreased in F_1T (1.2 %) and F_2T (1.7 %) amaranthus under enhanced UV-B exposure (Table 2). Spinach, however, showed reductions in Fm values in F_0T (1.3 %), F_1T (8.9 %), F_2T (5.1 %) and F_3T (5.3 %) under enhanced UV-B (Table 2). Fv increased significantly in F_0T (14.5 %) amaranthus and decreased significantly by 5.1, 15.8, 15.4 and 29.6 %, respectively, in F_0T, F_1T, F_2T, F_3T spinach compared with their controls (Table 2). (Table 2). Fv/Fm ratio reduced significantly at all nutrient levels in spinach with maximum reduction in F_3T (25.6 %) and minimum in F_0T (3.9 %), and in F_0T and F_1T amaranthus (Table 2).

Malondialdehyde content increased significantly under enhanced UV-B with highest percent increment in F_3T (54.8 and 57.7) and lowest in F_1T (16.8 and 25.9) amaranthus and spinach, respectively, compared with their controls (Fig. 3). Enhanced UV-B exposure led to significant reductions in chlorophyll content in F_0T (9.9 %), F_1T (5.4 %), F_2T (10.1 %) and F_3T (25.4 %) amaranthus, and F_2T (47.1 %) and F_3T (56.5 %) spinach, respectively (Fig. 3). Carotenoids increased under enhanced UV-B in amaranthus at all NPK levels, while spinach showed increments in F_0T and F_1T and reductions in F_2T and F_3T (Fig. 3). Enhanced UV-B exposure induced increments in UV-B-absorbing compounds in F_0T (86.3 %), F_1T (57.3 %), F_2T (19.6 %) and F_3T (129.1 %) amaranthus, while spinach exhibited increments in F_0T (54.4 %) and F_1T (33.5 %) and reduction in F_3T (41.1 %) (Fig. 4).

Ascorbic acid content increased under enhanced UV-B radiation with higher increase in amaranthus compared with spinach; however, spinach exhibited significant increments in F_0T (32.4 %), F_1T (8.0 %), F_2T (33.8 %), but insignificant in F_3T (13.3 %) plants (Fig. 5). Protein content declined under enhanced UV-B with maximum reduction in F_3T (64.6 and 67.2 %) and minimum in F_2T (30.9 and 39.8 %) amaranthus and spinach plants, respectively (Fig. 5). Total phenol content increased in F_1T (7.8 %) and F_3T (10.1 %) amaranthus under enhanced UV-B and reduced in spinach at all NPK levels with maximum in F_2T (44.7 %) (Fig. 5).

Superoxide dismutase activity increased in F_3T (215.9 %), but reduced in F_1T (12.2 %) and F_2T (32.7 %) amaranthus under enhanced UV-B exposure (Fig. 6). Percent reductions in SOD activities of F_0T, F_1T, F_2T and F_3T were 63.5, 44.9, 37.7 and 50.2, respectively, in spinach compared with their controls (Fig. 6). Enhanced UV-B led to increase in POX activity at all nutrient levels with maximum in F_2T and minimum in F_0T amaranthus plants (Fig. 6). POX activity increased in F_3T (43.5 %), but reduced in F_0T (30.1 %) and F_1T (64.4 %) spinach under enhanced UV-B (Fig. 6).

Both amaranthus and spinach showed reductions in total biomass with maximum in F_3T (47.9 and 43.2 %) and

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

Species	Factors	Nutrient (N)	Treatment (T)	N × T
Amaranthus	Photosynthesis	177.5***	103.8***	3.6*
	Stomatal conductance	153.1***	196.9***	85.0***
	Water use efficiency	15.1***	8.9**	6.0**
	Initial fluorescence	15.3***	0.040^{ns}	5.2*
	Fm	11.9***	4.3^{ns}	7.1**
	Fv	7.9**	7.5*	4.8*
	Fv/Fm	1399.1***	785.9***	76.0***
	MDA content	351.1***	1189.9***	93.9***
	Chlorophyll	2284.7***	1100.3***	81.2***
	Carotenoid	7549.3***	43917.7***	5209.2***
	UV-B-absorbing compounds	31.9***	998.7***	67.2***
	SOD	412.6***	1006.2***	5.7**
	POX	177.7***	180.5***	1.9^{ns}
	Ascorbic acid	67.6***	159.1***	12.5***
	Phenol	144.9***	77.6***	9.8**
	Protein	465.7***	949.1***	60.0***
	Biomass	556.4***	219.9***	1.7^{ns}
	RSR	4.9*	12.0**	6.3**
	Yield	4131.3***	2704.5***	128.0***
	Nitrogen use efficiency	18.6***	3.9^{ns}	1.6^{ns}
Spinach	Photosynthesis	10337.7***	1032.1***	296.3***
	Stomatal conductance	13157.2***	16734.7***	7439.7***
	Water use efficiency	266.7***	93.8***	28.5***
	Initial fluorescence	1198.3***	322.4***	55.4***
	Fm	286.9***	225.9***	23.8***
	Fv	1847.8***	936.5***	52.2***
	Fv/Fm	3558.5***	1460.3***	153.4***
	MDA content	1127.0***	7346.6***	269.2***
	Chlorophyll	189.1***	378.4***	125.3***
	Carotenoid	2458.9***	1984.3***	2669.9***
	UV-B-absorbing compounds	32.1***	65.9***	572.3***
	SOD	475.3***	5658.9***	210.1***
	POX	38.7***	54.4***	68.6***
	Ascorbic acid	3456.9***	150.4***	67.6***
	Phenol	107.5***	4605.7***	215.8***
	Protein	960.8***	225.8***	54.3***
	Biomass	937.8***	1112.2***	3.2^{ns}
	RSR	572.3***	1100.3***	271.6***
	Yield	1476.6***	486.4***	27.6***
	Nitrogen use efficiency	96.9***	180.8***	34.6***

Level of significance: *ns* not significant

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

minimum in F_1T (19.2 and 23.9 %) (Fig. 7). RSR was lower in F_0T (32.2 and 32.8 %), F_1T (25.2 and 62.9 %), F_2T (52.9 and 66.7 %) amaranthus and spinach under enhanced UV-B, while increased in F_3T amaranthus (33.8 %) and decreased in F_3T spinach (19.2 %) (Fig. 7). Enhanced UV-B exposure led to reduction in yield with maximum in F_3T (41.2 %) of amaranthus and F_2T (28.2 %) of spinach (Fig. 8). Minimum reduction in yield was observed in F_1T (8.1 %) for both the species (Fig. 8).

Enhanced UV-B radiation led to insignificant reduction in NUE in amaranthus plants (Fig. 7), while significant reductions in F_0T (44.2 %), F_1T (9.6 %) and F_2T (31.4 %) spinach compared with their controls (Fig. 8).

The control and enhanced UV-B treated values of various parameters under different NPK levels of spinach and amaranthus were subjected to a principal component analysis (Fig. 9). In both the species, first two components accounted for the majority of variations in the data set. In

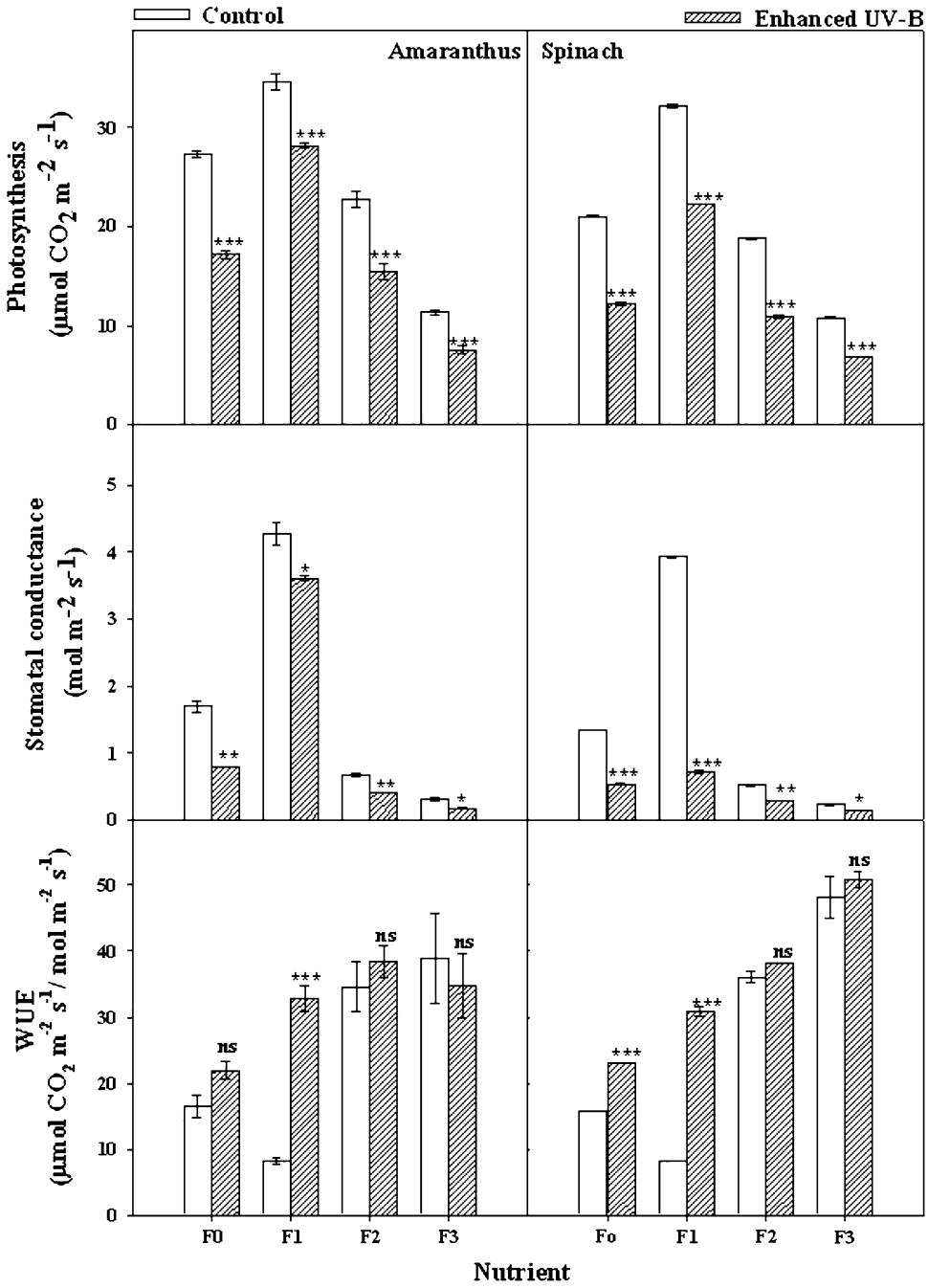

Fig. 2 Effects of enhanced UV-B treatment on photosynthesis, stomatal conductance and water use efficiency of amaranthus and spinach plants under varying nutrient levels. Values are mean ± 1SE. Level of significance between control and enhanced UV-B treated plants: *ns* not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. F_0: recommended dose of NPK, F_1: 1.5× recommended dose of NPK, F_2: 1.5× recommended dose of N, F_3: 1.5× recommended dose of K

spinach, 39.6 % variations could be explained by the first principal component (PC1) and 23.6 % by the second principal component (PC2). Similarly in amaranthus, 47.1 % variations could be explained by PC1 and 21.2 % by the PC2. It is clear from Fig. 9 that spinach with high values of chlorophyll, SOD and phenol, and amaranthus with high values of chlorophyll, SOD and phenol are located towards the positive end of the PC1 axis, and spinach with high values of protein, gs and ascorbic acid and amaranthus with high values of protein and gs are located towards the positive end of PC2 axis. Membrane damage, carotenoid content, Fv/Fm and UV-B-absorbing compounds were most commonly affected parameters in both the species, while specific sensitivity was shown by ascorbic acid content in amaranthus. Protein, chlorophyll and gs were moderately affected by enhanced UV-B under different NPK levels in both the species. SOD and phenol in spinach are the other parameters moderately affected by enhanced UV-B. Phenol and SOD in amaranthus are the least affected parameters.

Table 2 Effects of enhanced UV-B on chlorophyll fluorescence yields of amaranthus and spinach plants under varying nutrients

Plants	Nutrient	Treatments	Initial fluorescence	Maximum fluorescence	Variable fluorescence	Fv/Fm
Amaranthus	F_0	C	343 ± 4.0	1041 ± 8.7	698 ± 4.6	0.673 ± 0.001
		T	365.5 ± 6.6*	1164.5 ± 33.2*	799 ± 26.5*	0.651 ± 0.002*
	F_1	C	374.5 ± 2.6	1148 ± 13.8	773.5 ± 11.3	0.686 ± 0.003
		T	356 ± 5.8*	1134.5 ± 19.3*	778.5 ± 25.1^{ns}	0.656 ± 0.002*
	F_2	C	388 ± 9.8	1209 ± 24.5	821.5 ± 14.7	0.679 ± 0.001
		T	387 ± 4.6^{ns}	1188.5 ± 7.2**	815.5 ± 11.3^{ns}	0.623 ± 0.003^{ns}
	F_3	C	382 ± 2.9	1175.5 ± 2.0	793.5 ± 0.87	0.575 ± 0.002
		T	376 ± 0.58^{ns}	1190 ± 10.6*	814 ± 111.3^{ns}	0.483 ± 0.003^{ns}
Spinach	F_0	C	354 ± 1.3	1036 ± 3.0	682 ± 4.3	0.658 ± 0.002
		T	376 ± 1.2***	1023 ± 2.8***	647 ± 4.0***	0.632 ± 0.002***
	F_1	C	381 ± 3.4	1071 ± 3.7	691 ± 7.1	0.644 ± 0.004
		T	394 ± 3.0***	976 ± 6.5*	582 ± 3.46**	0.596 ± 0.0003**
	F_2	C	409 ± 2.7	952 ± 1.2	543 ± 3.81	0.570 ± 0.003
		T	444 ± 2.4**	903.6 ± 6.9*	459.6 ± 4.9***	0.509 ± 0.004***
	F_3	C	491 ± 4.7	948.7 ± 7.4	457.7 ± 2.7	0.483 ± 0.0009
		T	576 ± 3.9***	898 ± 3.6**	322 ± 0.35***	0.359 ± 0.003***

Values are mean ± 1SE

Level of significance between control and enhanced UV-B treated amaranthus and spinach plants: *ns* not significant. F_0: recommended dose of NPK, F_1: 1.5× recommended dose of NPK, F_2: 1.5× recommended dose of N, F_3: 1.5× recommended dose of K

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Discussion

Photosynthetic rate reduced significantly at all concentrations and combinations of NPK under enhanced UV-B, with more reduction in spinach compared with amaranthus. Allen et al. (1998) reported that the lowering in photosynthetic rate under enhanced UV-B is related to its negative effects on photosynthetic apparatus and/or on Calvin cycle enzymes, CO_2 diffusion changes, etc. An amendment of 1.5× NPK provided best protection to photosynthetic system of both the species under enhanced UV-B stress, and the magnitude of protection was higher in amaranthus compared with spinach. Better performance of amaranthus, a C_4 species under enhanced UV-B at 1.5× NPK is related to its capability to capitalize N, when abundant. C_4 plants achieve higher photosynthetic capacity than C_3 plants due to lack of photorespiration, thus enabling the plant to achieve high NUE (Furbank et al. 2000). The photosynthetic capacity of leaves is related to the nitrogen content primarily due to the proteins of the Calvin cycle, which represent the majority of leaf nitrogen (Evans 1989). In the present study, amaranthus showed higher NUE and photosynthesis rate compared with spinach at all NPK levels.

Variation in photosynthesis is also dependent on the ability of a leaf to draw down CO_2 within the leaf. Lower stomatal conductance in both the species suggests a direct impact of UV-B on stomatal closure (Day and Vogelmann 1995) and stomatal limitation accounting for the decline in photosynthesis. A non-linear relationship between stomatal conductance and photosynthetic capacity in spinach observed at 1.5× NPK under enhanced UV-B suggests that decline in photosynthesis is not fully accounted due to stomatal closure. Conner and Zangori (1998) showed that plants supplied with more than optimum nitrogen, compensated for, or protected themselves from detrimental effects of UV-B on photosynthesis and stomatal conductance. F_2C and F_3C plants of both the species with low photosynthesis and stomatal conductance had higher intrinsic WUE than other treatments with proportionally higher Ps and gs, which further varied insignificantly under enhanced UV-B. F_1T amaranthus and F_0T and F_1T spinach plants with higher intrinsic WUE had the capacity to compensate the photosynthetic loss associated with reduced stomatal conductance (Gilbert et al. 2011). Chlorophyll fluorescence has been widely used to detect stress-induced perturbations in the photosynthetic apparatus. Light dependent inactivation of PSII reaction centres is associated with a decline in Fm and Fv/Fm and with an increase of initial fluorescence yield as observed in spinach under enhanced UV-B at all NPK levels and in amaranthus at recommended NPK. Increase in Fv of F_0T amaranthus may be ascribed to the large increase in Fm. Reductions in Fm values of F_1T and F_2T amaranthus and at all NPK levels in spinach denote reduced electron transfer capacity under UV-B stress. An increase in the rate constant of non-radiative energy dissipation leads to a decrease in both

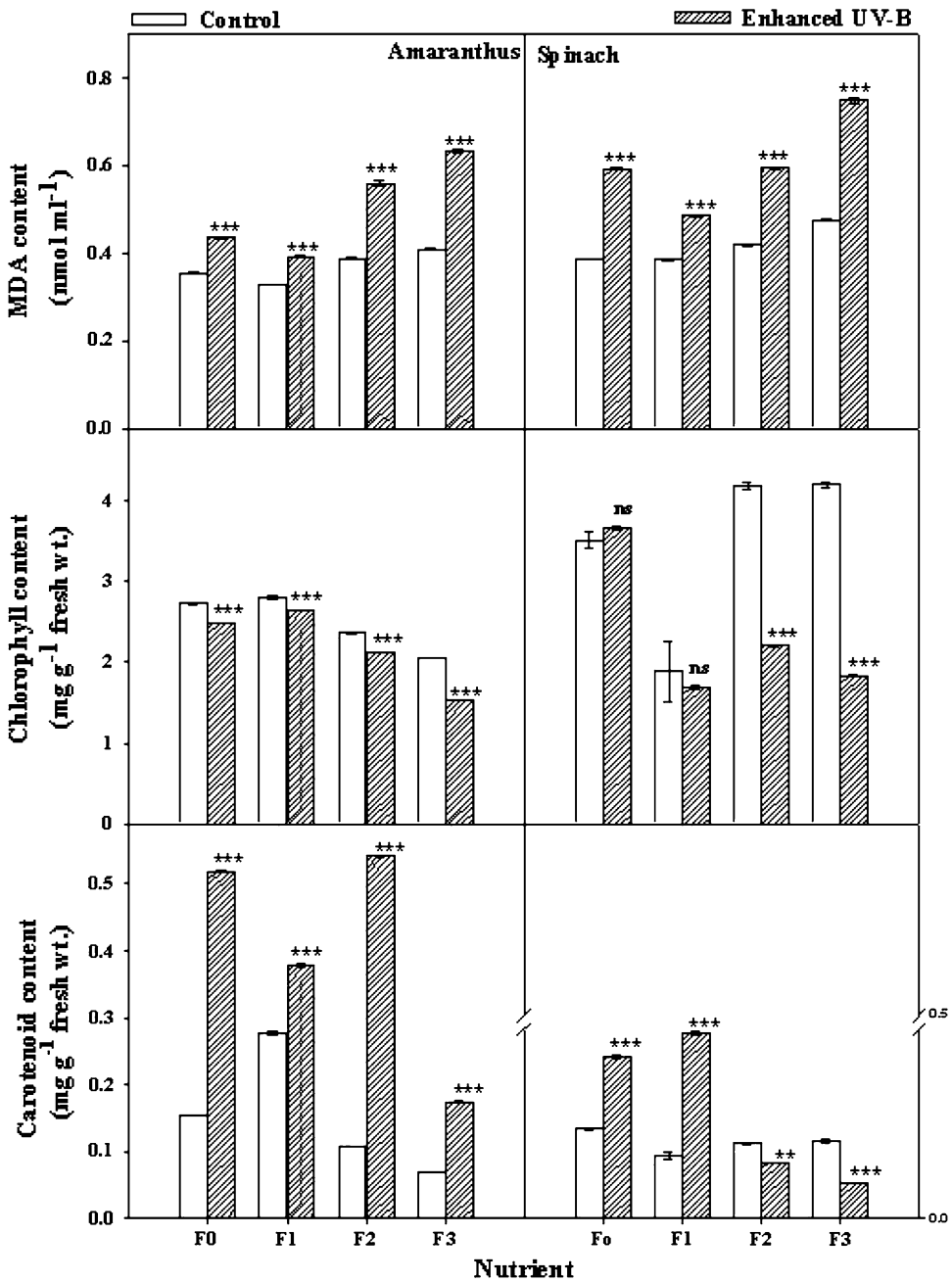

Fig. 3 Effects of enhanced UV-B treatment on MDA, total chlorophyll and carotenoid content of amaranthus and spinach plants under varying nutrient levels. Values are mean ± 1SE. Level of significance between control and enhanced UV-B treated plants: *ns* not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. F_0: recommended dose of NPK, F_1: 1.5× recommended dose of NPK, F_2: 1.5× recommended dose of N, F_3: 1.5× recommended dose of K

initial fluorescence and Fm as is observed in F_1T amaranthus. Such an increase in thermal de-excitation could function as an overflow valve to allow for nondestructive dissipation of excess excitation energy, and may therefore be viewed as a potentially protective process rather than as an indication of damage (Kyle et al. 1984). Pattern of change in Fv/Fm was different between the two species with amaranthus having higher average values than spinach at all nutrient levels. Reductions in Fv/Fm can be due to slow quenching and photodamage to PSII reaction centres, which may reduce the maximum quantum efficiency of PSII photochemistry (Baker and Rosenqvist 2004). It is probable that the linear flow of electron beyond PSII is also decreased under enhanced UV-B radiation, leading to a reduced proportion of e^-(s) available for the Calvin cycle. The reduction in maximum e^- transport rate leads to decrease in RuBP regeneration (Onoda et al. 2005), and thus, decreases the maximum rate of Rubisco carboxylation, thereby lowering the photosynthetic performance even more. Additional soil NPK supply increased the photosynthetic capacity manifested by increase in the light saturated rate of photosynthesis and the apparent quantum yield of PSII as recorded in the present study. Also, insignificant variation in Fv/Fm of F_2T and F_3T amaranthus

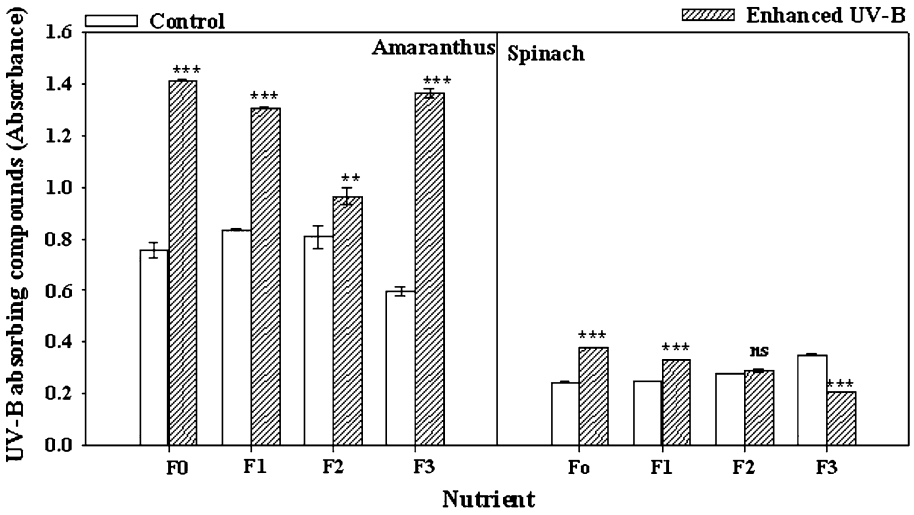

Fig. 4 Effects of enhanced UV-B treatment on UV-B-absorbing compounds of amaranthus and spinach plants under varying nutrient levels. Values are mean ± 1SE. Level of significance between control and enhanced UV-B treated plants: *ns* not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. F_0: recommended dose of NPK, F_1: 1.5× recommended dose of NPK, F_2: 1.5× recommended dose of N, F_3: 1.5× recommended dose of K

suggests that the primary damaging effect of UV-B on photosynthesis may have resulted from the decreased Calvin cycle enzyme activity or content or transcription of photosynthetic genes and not due to PSII damage. Maximum reduction in Fv/Fm ratio was observed in F_3T spinach grown in K-amended soil.

Enhanced UV-B radiation induced oxidative stress in both the species as observed by increments in MDA content, which was recorded maximum in F_3T plants grown at 1.5× K and minimum in 1.5× NPK, suggesting that NPK at higher dose reduced the oxidative stress more than any other amendments. This observation proves our proposed hypothesis that the oxidative stress induced in plants under enhanced UV-B will be reduced maximally at 1.5× NPK. Changes in the photochemistry of the chloroplasts under enhanced UV-B result in dissipation of excess light energy, resulting in the generation of reactive oxygen species (ROS) (Peltzer et al. 2002). Spinach showed higher MDA content than amaranthus as ROS production and oxidative damage are expected to be lower in C_4 plants, mostly due to an absence of photorespiration (Uzilday et al. 2012). Huang et al. (2004) also reported an increase in MDA content of N-deficient rice plants. Reduction in CO_2 assimilation capacity under enhanced UV-B was further enhanced by low N leading to an over reduction of photosynthetic electron chain and leakage of electron thus enhancing the ROS formation.

The decline in chlorophyll content of both the species under enhanced UV-B at least in part, can be due to LPO in the chloroplast membranes. The photoreduction of protochlorophyllide to chlorophyllide by protochlorophyllide oxidoreductase is one of the possible targets of UV-B (Marwood and Greenberg 1996). Because this reaction is light driven, it is possible that UV-B can damage this enzyme resulting in lower rate of chlorophyll accumulation (Marwood and Greenberg 1996). Also, chlorophyll is stabilized by association with proteins. UV-B radiation causes a downregulation of the expression of genes, which encode for chlorophyll a/b binding proteins (Casati and Walbot 2003). Degradation of chlorophyll occurred maximally in F_3T spinach and minimum in F_1T plants. In general, chlorophyll content increases with increasing N supply, but at higher than recommended NPK chlorophyll content was minimum in spinach both in control and enhanced UV-B treatment. Moreover, if Liebig's law of the minimum, which states that 'the environmental resource present in the least amount will determine plant growth', applies, then positive response to higher N may not necessarily occur. Carotenoids increased at all NPK levels in amaranthus, but only in F_0T and F_1T spinach under enhanced UV-B radiation. The decreased carotenoid levels in F_2T and F_3T imply increased sensitivity of spinach to photo-oxidative damage by UV-B (Rau et al. 1991). Ravindran et al. (2001) reported reduction in carotenoids by 13.3 % in *Suaeda maritima* under 12.2 kJ m^{-2} day^{-1} UV-B radiations. Becatti et al. (2009), however, reported accumulation of carotenoids (52.6 %) in tomato plants under UV-B radiation.

Ascorbic acid increased in both the species under enhanced UV-B (except F_3T spinach) and the increment was more pronounced in amaranthus. Increase in ascorbic acid in response to enhanced UV-B was reported earlier (Galatro et al. 2001). Higher nutrient availability did not change the magnitude of increase in ascorbic acid under enhanced UV-B stress. Ascorbic acid reacts directly with ROS and also reduces the oxidized form of α-tocopherol (Xu et al. 2008), thus prevents oxidative stress. SOD activity decreased under enhanced UV-B (except F_3T amaranthus), while POX increased in amaranthus at all NPK levels, but decreased in spinach except F_3T. This trend clearly shows variations in POX activities under enhanced UV-B between the species and nutrient

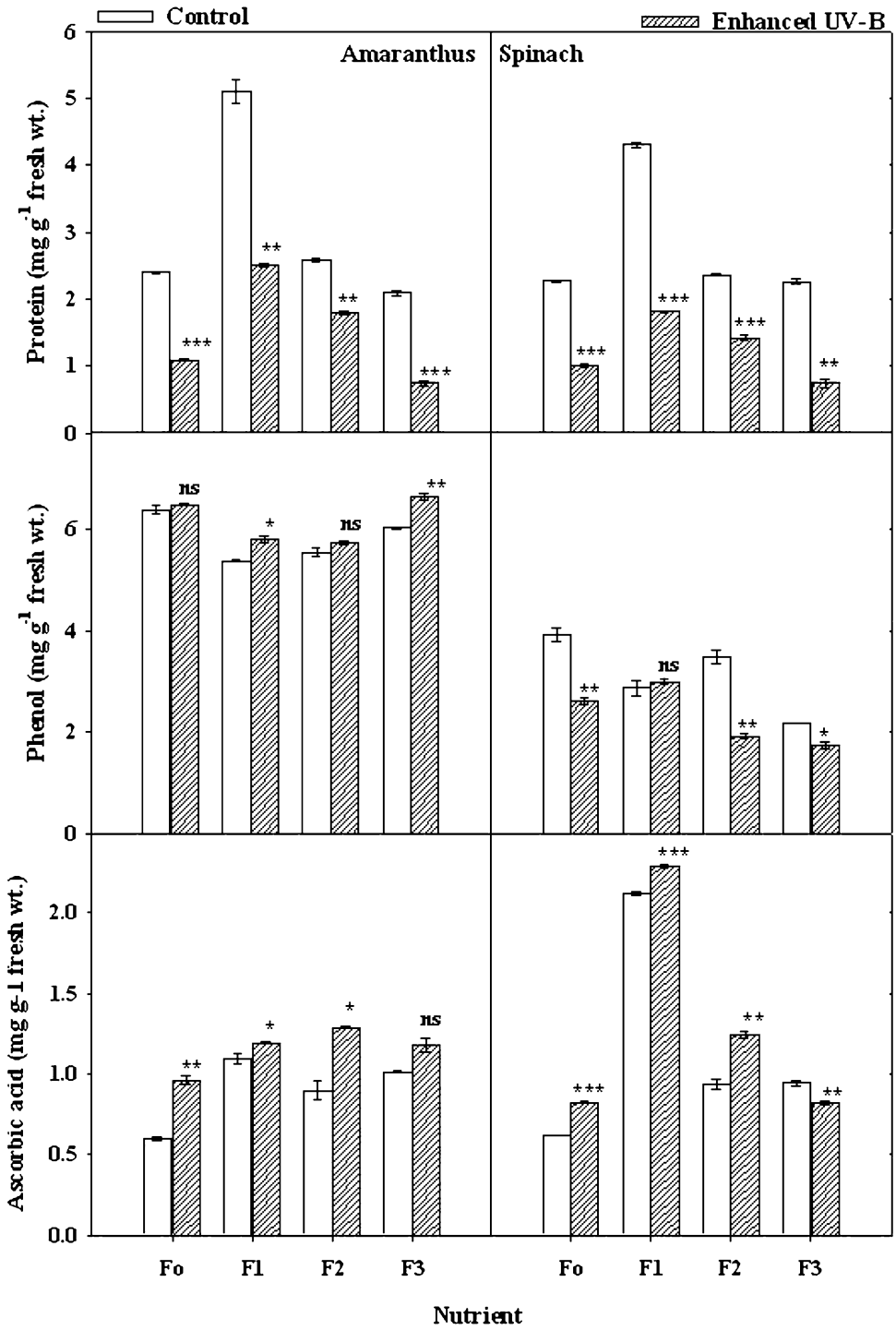

Fig. 5 Effects of enhanced UV-B treatment on protein, phenol and ascorbic acid content of amaranthus and spinach plants under varying nutrient levels. Values are mean ± 1SE. Level of significance between control and enhanced UV-B treated plants: *ns* not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. F_0: recommended dose of NPK, F_1: 1.5× recommended dose of NPK, F_2: 1.5× recommended dose of N, F_3: 1.5× recommended dose of K

treatment. Higher induction in POX in amaranthus suggests greater scavenging of H_2O_2 which make these plants less sensitive to UV-B compared with spinach.

Enhanced UV-B radiation led to reduction in protein content with higher reduction in spinach. Despite maximum reduction in total protein in F_3T plants, the biochemical evaluations showed that the measured antioxidative enzymes increased. This suggests that the reduction in protein content is mainly related to primary metabolism or structural protein. Higher reductions in protein content were observed in F_1T plants of both the species. Higher N availability to plants grown in 1.5× NPK-amended soil prompted the plants to invest a greater proportion of carbon in protein such as different enzymes which have been identified as a target of UV-B. Even then, the average protein values of control and enhanced UV-B treated plants at 1.5× NPK were higher than the other

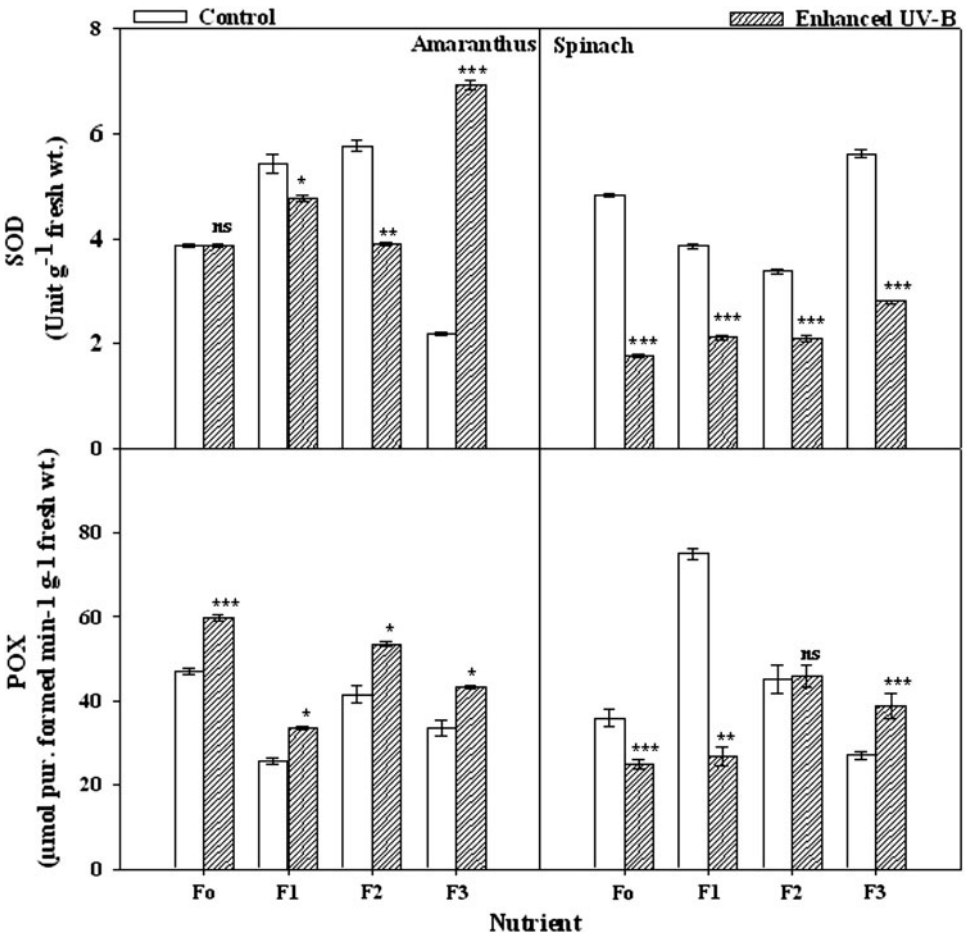

Fig. 6 Effects of enhanced UV-B treatment on SOD and POX activities of amaranthus and spinach plants under varying nutrient levels. Values are mean ± 1SE. Level of significance between control and enhanced UV-B treated plants: *ns* not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. F_0: recommended dose of NPK, F_1: 1.5× recommended dose of NPK, F_2: 1.5× recommended dose of N, F_3: 1.5× recommended dose of K

treatments. Phenol content either did not change (F_1T) or reduced in spinach while increased in amaranthus plants under enhanced UV-B (F_1T and F_3T). As an adaptation to UV-B, phenols can reduce the amount of UV-B reaching the photosynthetic tissues of the leaves (mesophyll), where UV-B can be damaging. Phenylalanine ammonia lyase (PAL) mediating the initial step of phenylpropanoid metabolism can liberate ammonium ions from phenylalanine and skeletons of t-cinnamate could contribute to increase in different phenolic metabolites under low N availability (Stewart et al. 2001), but excess of NPK also led to a similar response under enhanced UV-B in the present study. In F_1T and F_3T amaranthus plants, reductions in protein were followed by increments in total phenolics, suggesting that there exist an inverse correlation between phenol and protein. Ilvessalo and Tuomi (1989) proposed that relative abundance of carbon and mineral nutrients especially N influences the rate at which the substrate, i.e. phenylalanine is diverted to protein synthesis. It seems that higher nutrient also increased carbon, which led to N dilution, and hence, protein declined with consequent increase in total phenolics as is observed in F_1T amaranthus.

Production of UV-B-absorbing compounds is a known defence strategy against the deleterious effects of UV-B radiation (Meijkamp et al. 1999). Amaranthus showed greater ability to synthesize UV-B-absorbing compounds than spinach, resulting in a higher UV-B screening capacity. The improved screening capacity ought to result in a stress release on photosynthetic performance. Although, F_0T of both the species having higher induction of UV-B-absorbing compounds than F_1T, showed more decline in photosynthesis than F_1T. Higher increase in specific leaf weight of F_1T amaranthus (10.2 %; data not shown) might have reduced transmittance of UV-B towards photosynthesizing cells, thus effectively protected the photosynthetic machinery. Maximum increase in UV-B-absorbing compounds was recorded for F_3T amaranthus plants. But despite this increase, the negative effects of UV-B on the photosynthetic apparatus could not be prevented. UV-B-absorbing compounds reduced under enhanced UV-B in F_3T spinach showed highest sensitivity. Plants that do not show induction in UV-B-absorbing compounds under UV-B are sensitive (Xu et al. 2010).

Reductions in total biomass and yield were observed in both the species under enhanced UV-B exposure at all NPK levels with minimum at 1.5× NPK. Smith et al. (2000) suggested that reduced biomass accumulation is

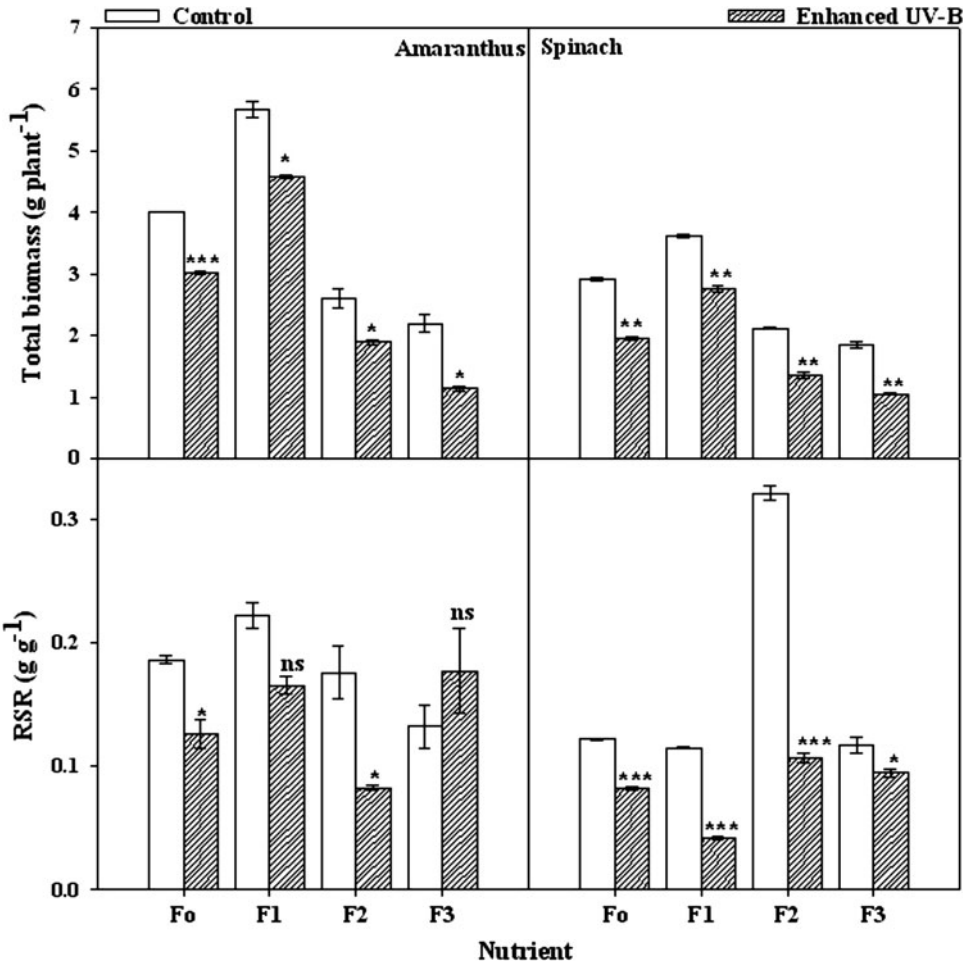

Fig. 7 Effects of enhanced UV-B treatment on total biomass and RSR of amaranthus and spinach plants under varying nutrient levels. Values are mean ± 1SE. Level of significance between control and enhanced UV-B treated plants: *ns* not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. F_0: recommended dose of NPK, F_1: 1.5× recommended dose of NPK, F_2: 1.5× recommended dose of N, F_3: 1.5× recommended dose of K

often a reliable indication of plant's sensitivity to UV-B radiation, as it represents the cumulative effects of damaged or inhibited physiological functions. Amaranthus may have invested more N into new leaf production under 1.5× NPK than spinach, and therefore has a higher whole plant carbon gain compared with later species. Alleviation of UV-B stress under higher availability of NPK was more in amaranthus than in spinach. Reduction in biomass was accompanied by substantial modification in the partitioning of biomass in above and below ground plant parts. Reduction in RSR values of all the spinach plants, and F_0T, F_1T and F_2T amaranthus plants under enhanced UV-B showed that UV-B treated plants retained more carbon for repair of above ground parts under different NPK combination compared with individual nutrient. The observed increase in RSR of F_3T amaranthus was due to increase in partitioning of resources to roots to stimulate uptake of nutrients under low N and P availability.

In the present study, plant species differed in their NUE, which was higher in amaranthus compared with spinach under enhanced UV-B exposure. Amaranthus did not show significant difference in NUE, but spinach showed significant decline under enhanced UV-B except in F_3T. This suggests that amaranthus maintained high NUE, and hence, showed greater protection under enhanced UV-B exposure compared with spinach. NUE is related to inclusion of N in the enzymes of carboxylation and metabolism (Brown 1978). It appears that the ability of amaranthus to maximize photosynthetic carbon uptake for a given unit investment of N is higher compared with spinach. This observation is consistent with the hypothesis that the plant species showing higher NUE will show lower sensitivity to enhanced UV-B.

In our study, the important physiological and biochemical trait variances were extracted into two principal components by a PCA approach. The presence of most sensitive and moderately sensitive parameters was recorded for both the species, but least sensitive parameter was only observed for amaranthus. The analysis of moderately sensitive parameters suggest that impairment in the protection provided by UV-B-absorbing compounds, carotenoid and ascorbic acid content led to membrane damage and inhibition of PSII reaction centre. Amaranthus showed lower sensitivity due to higher phenol accumulation and SOD activity.

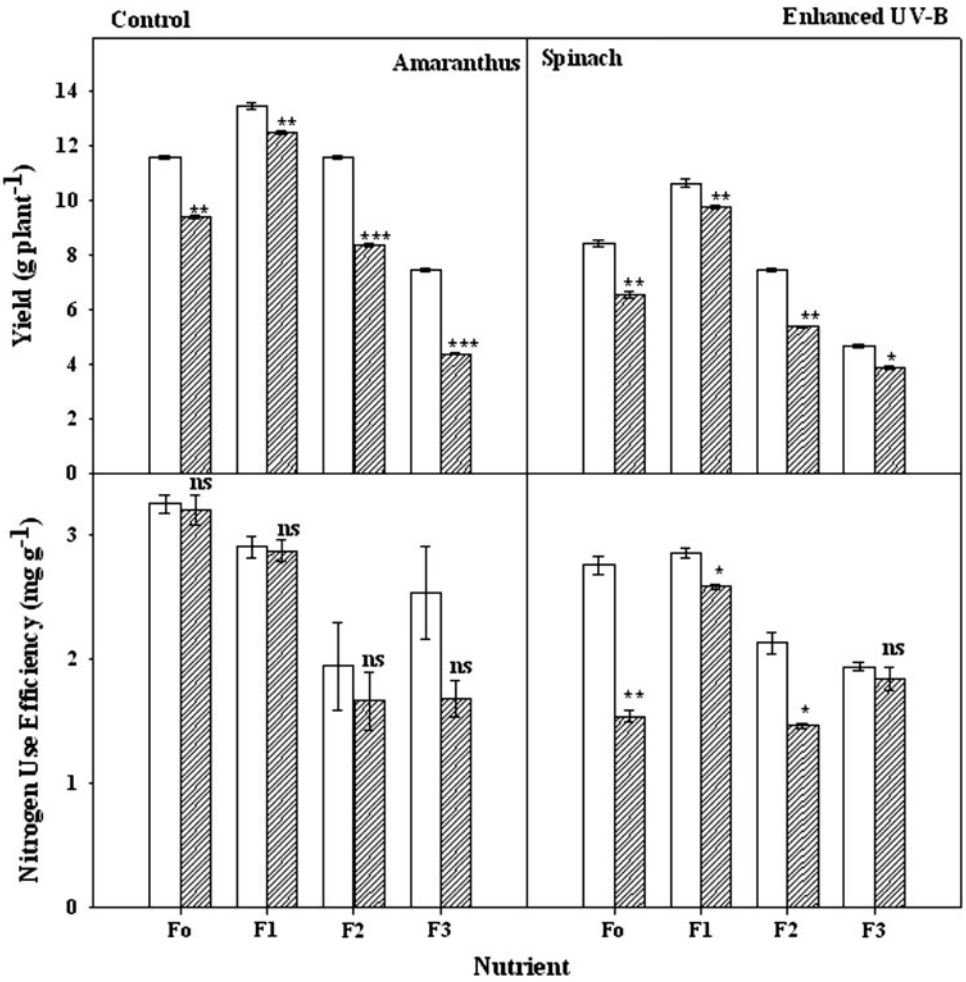

Fig. 8 Effects of enhanced UV-B treatment on yield and nitrogen use efficiency of amaranthus and spinach plants under varying nutrient levels. Values are mean ± 1SE. Level of significance between control and enhanced UV-B treated plants: *ns* not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. F_0: recommended dose of NPK, F_1: 1.5× recommended dose of NPK, F_2: 1.5× recommended dose of N, F_3: 1.5× recommended dose of K

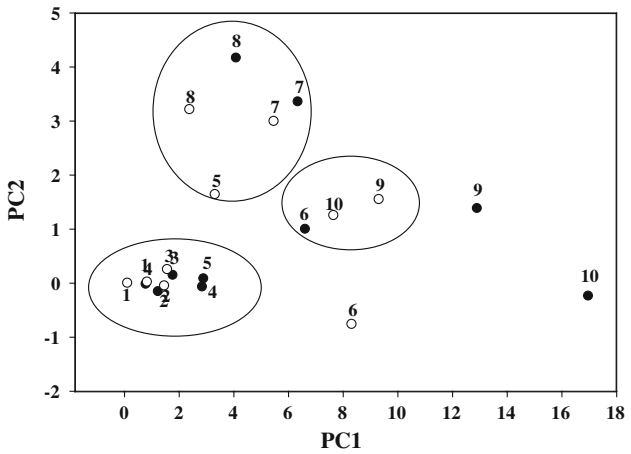

Fig. 9 The principal component analysis (PCA) of spinach and amaranthus. The *scatter plot* of both the species on the PC1 and PC2 side. Open (*white circle*) and closed (*black circle*) symbols denote amaranthus and spinach, respectively, under ambient and enhanced UV-B radiation at varying NPK levels. Each *symbol* represents the mean values for the PC scores of the three replicates of each parameter. For the convenience, the parameters are denoted by *numbers*, carotenoid (*1*), MDA content (*2*), Fv/Fm (*3*), UV-B-absorbing compounds (*4*), ascorbic acid (*5*), chlorophyll (*6*), protein (*7*), gs (*8*), SOD (*9*), phenol (*10*)

Conclusions

The present investigation revealed that the sensitivity of spinach and amaranthus plants to enhanced UV-B radiation varied with the availability of different concentrations and combinations of NPK. The varying sensitivity of both the plants depends on their ability to counter the oxidative stress generated under enhanced UV-B exposure. Photosynthetic performance of amaranthus was better than spinach under enhanced UV-B radiation at all NPK levels. Quantum yield declined in both the species under enhanced UV-B stress, with maximum decline under low N and P availability and minimum at 1.5× NPK. Enhanced UV-B led to increase in MDA content at all NPK levels with minimum at 1.5× NPK. Spinach showed larger increase in MDA content compared with amaranthus. Correspondingly, higher ascorbic acid content and reduction in POX activity were recorded in later compared with former species. Amaranthus showed higher induction in UV-B-absorbing compounds denoting higher UV-B screening capability compared with spinach. Amaranthus also showed higher NUE compared with spinach at all NPK

levels. It may be concluded that spinach is more sensitive compared with amaranthus due to lower antioxidative capacity, UV-B screening capability and more disturbances in photosynthetic performances. Both the species showed lowest sensitivity against UV-B at 1.5× NPK. PCA score also revealed that the sensitivity of both the species to UV-B is mainly ascribed to membrane damage and inhibition of PSII reaction centre. Lower sensitivity of amaranthus to UV-B at all NPK levels was due to more accumulation of phenol and SOD activity. More studies having mechanistic approach are required to evaluate the inter species response to UV-B at varying soil NPK levels.

Acknowledgments The authors thank the Head of Department of Botany, Banaras Hindu University for providing necessary laboratory facilities. The authors are also acknowledging the authorities of the Banaras Hindu University and Council of Scientific and Industrial Research (CSIR), New Delhi for providing financial assistance. And the special thanks are to the reviewers for their valuable suggestions and corrections on the article.

References

Adamse WW, Britz SJ (1992) Amelioration of UV-B damage under high irradiance. I: role of photosynthesis. Photochem Photobiol 56:645–650

Allen DJ, Nogues S, Baker NR (1998) Ozone depletion and increased UV-B radiation: is there a real threat to photosynthesis? J Exp Bot 49:1775–1788

Baker NR, Rosenqvist E (2004) Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. J Exp Bot 55:1607–1621

Balakumar T, Hanibabu VV, Paliwal K (1993) On the interaction of UV-B radiation (280–315 nm) with water stress in crop plants. Physiol Plant 87:217–222

Becatti E, Petroni K, Giuntini D, Castagn A, Calvenzani V, Serra G, Mensauli-Sodi A, Tonelli C, Ranieri A (2009) Solar UV-B radiation influences carotenoid accumulation of tomato fruit through both ethylene-dependent and independent mechanisms. J Agric Food Chem 57:10979–10989

Björn LO (1996) Effects of ozone depletion and increased UV-B on terrestrial ecosystems. Int J Environ Stud 51:217–243

Bray HG, Thorpe WY (1954) Analysis of phenolic compounds of interest in metabolism. Meth Biochem Anal 1:27–52

Britton C, Mehley AC (1955) Assay of catalase and peroxidase. In: Colowick SP, Kalpan NO (eds) Method in Enzymology. Academic Press Inc., New York, p 764

Brown RH (1978) A difference in N use efficiency in C_3 and C_4 plants and its implications in adaptation and evolution. Crop Sci 18:93–98

Cabrera S, Bozzo S, Fuenzalida H (1995) Variations in UV radiation in Chile. J. Photochem Photobiol 28:137–142

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

Casati P, Walbot V (2003) Gene expression profiling in response to ultraviolet radiation in maize genotypes with varying flavonoid content. Plant Physiol 132:1739–1754

Conner JK, Zangori LA (1998) Combined effects of water, nutrient and UV-B stress on female fitness in *Brassica* (Brassicaceae). Am J Bot 85:925–931

Day TA, Vogelmann TC (1995) Alterations in photosynthesis and pigment distribution in pea leaves following UV-B exposure. Physiol Plant 94:433–440

Duxbury AC, Yentsch CS (1956) Plankton pigment monographs. J Mar Res 15:19–101

Evans JR (1989) Photosynthesis and nitrogen relationship in leaves of C_3 plants. Oecologia 78:9–19

Fridovich I (1974) Superoxide dismutase. Adv Enzymol 41:35–97

Furbank RT, Hatch MD, Jenkins CLD (2000) C4 photosynthesis: mechanism and regulation. In: Leegood RC (ed) Photosynthesis: Physiology and Metabolism. Kluwer Acedemic Publications, Dordrecht/Boston/London, pp 435–457

Galatro A, Simontacchi M, Puntarulo S (2001) Free radical generation and antioxidant content in chloroplasts from soybean leaves exposed to ultraviolet-B. Physiol Plant 113:564–570

Gilbert ME, Zwieniecki MA, Hobbrook NM (2011) Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. J Exp Bot 62(8):2875–2887

Green AES, Cross KR, Smith LA (1980) Improved analytical characterization of ultraviolet skylight. Photochem Photobiol 31:59–65

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198

Huang Z-A, Jiang D-A, Yang Y, Sun J-W, Jin S-H (2004) Effects of nitrogen deficiency on gas exchange, chlorophyll fluorescence, and antioxidant enzymes in leaves of rice plants. Photosynthetica 42:357–364

Hunt R (1982) Plant growth analysis. University Press, Baltimore, USA

Ilvessalo H, Tuomi J (1989) Nutrient availability and accumulation of phenolic compounds in the brown alga *Fucus vesiculosus*. Mar Biol 101:115–119

Imbrie CW, Murphy TM (1982) UV-action spectrum (254–405 nm) for inhibition of a K-stimulated adenosine triphosphatase from the plasma membrane of *Rosa damascene*. Photochem Photobiol 36:537–542

Keller T, Schwager H (1977) Air pollution and ascorbic acid. Eur J Plant Pathol 7:338–350

Krizek DT, Mirecki RM, Britz SJ (1997) Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cucumber. Physiol Plant 100:886–893

Kyle DJ, Onad I, Arntzen CJ (1984) Membrane protein damage and repair: selective loss of quinine-protein fraction in chloroplast membranes. Proc Nat Acad Sci USA 81:4070–4074

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193: 265–275

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

Marwood CA, Greenberg BM (1996) Effect of supplementary UV-B radiation on chlorophyll synthesis and accumulation of photosystems during chloroplast development in *Spirodela oligorrhiza*. Photochem Photobiol 64:664–670

Meijkamp B, Aerts R, Van de Staaij J, Tosserams M, Ernst W, Rozema J (1999) Effects of UV-B on secondary metabolites in plants. In: Rozema J (ed) Stratospheric Ozone Depletion: the Effects of Enhanced UV-B Radiation on Terrestrial Ecosystems. Backhuys Publishers, Leiden, pp 71–99

Mirecki RM, Teramura AH (1984) Effects of ultraviolet-B irradiance on soybean. The dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. Plant Physiol 74:475–480

Moll RH, Kamprath EJ, Jackson W (1982) Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agronomy J 74:562–564

Murphy TM (1983) Membranes as targets of ultraviolet radiation. Physiol Plant 58:381–388

Nayyar H (2003) Accumulation of osmolytes and osmotic adjustment in water-stressed wheat (*Triticum aestivum*) and maize (*Zea mays*) as affected by calcium and its antagonists. Environ Exp Bot 50:253–264

Onoda Y, Hikosaka K, Hirose T (2005) Seasonal change in the balance between capacities of RuBP carboxylation and RuBP regeneration affects CO2 response of photosynthesis in P*olygonum cuspidatum*. J Exp Bot 56:755–763

Peltzer D, Dreyer E, Polle A (2002) Differential temperature dependencies of antioxidative enzymes in two contrasting species. Plant Physiol Biochem 40:141–150

Predieri S, Norman HA, Krizek DT, Pillai P, Mirecki RM, Zimmerman RH (1995) Influence of UV-B radiation on membrane lipid composition and ethylene evolution in cv. Doyenne d'hiver pear shoots growth in vitro under different photosynthetic photon fluxes. Environ Exp Bot 35:151–160

Rau W, Seigner L, Schrott EL (1991) The role of carotenoids in photoprotection against harmful effects of UV-B radiation. Biol Chem Hoppe Seyler 372:539

Ravindran KC, Maheskumar 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

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 26:3433–3440

Singh S, Kumari R, Agrawal M, Agrawal SB (2010) Growth, yield and tuber quality of *Solanum tuberosum* L. under supplemental ultraviolet-B radiation at different NPK levels. Plant Biol. doi: 10.1111/j.1438-2010.00395.x

Smith JL, Burritt 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 88:1057–1063

Stewart AJ, Chapman W, Jenkins GI, Graham T, Martin T, Crozier A (2001) The effect of nitrogen and phosphorus deficiency on flavonol accumulation in plant tissue. Plant Cell Environ 24:1189–1197

Uzilday G, Turkan I, Sekmen AH, Ozgur R, Karakaya HC (2012) Comparison of ROS formation and antioxidant enzymes in *Cleome gynandra* (C_4) and *Cleome spinosa* (C_3) under drought stress. Plant Sci 182:59–70

Visser AJ, Tossermans M, Groen MW, Magendans GWH, Rozema J (1997) The combined effects of CO_2 concentration and solar UV-B radiation on faba bean grown in open top chambers. Plant Cell Environ 20:189–199

Ward JK, Tissue DT, Thomas RB, Strain BR (1999) Comparative responses of model C_3 and C_4 plants to drought in low and elevated CO_2. Glob Change Biol 5:857–867

Xu C, Natarajan S, Sullivan JH (2008) Impact of solar ultraviolet-B radiation on the antioxidant defense system in soybean lines differing in flavonoid contents. Environ Exp Bot 63:39–48

Xu X, Zhao H, Zhang X, Hänninen H, Korpelainen H, Li C (2010) Different growth sensitivity to enhanced UV-B radiation between male and female *Populus cathayana*. Tree Physiol 30:1489–1498