

# Impact of ultraviolet-B radiation on photosynthetic capacity, antioxidative potential and metabolites in *Solanum tuberosum* L. under varying levels of soil NPK

Suruchi Singh · Madhoolika Agrawal ·  
S. B. Agrawal

Received: 23 October 2012/Revised: 12 March 2014/Accepted: 14 March 2014/Published online: 5 April 2014  
© Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Kraków 2014

**Abstract** The impact of supplemental ultraviolet-B (sUV-B; 280–315 nm;  $+7.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ ) radiation was studied on various physiological parameters, antioxidative potential and metabolites of *Solanum tuberosum* L. cv. Kufri Badshah plants under varying levels of soil NPK. The N, P and K treatments were: the recommended dose of N, P and K; 1.5 times the recommended dose of N, P and K; 1.5 times the recommended dose of N and 1.5 times the recommended dose of K. The recommended NPK level provided maximum protection to photosynthetic assimilation under sUV-B radiation, while stomatal conductance was best at 1.5 times the recommended NPK. Carbon dioxide assimilation declined maximally at 1.5 times the recommended N/K under sUV-B radiation. Plants grown at the recommended NPK and 1.5 times the recommended NPK levels showed higher superoxide dismutase, peroxidase and ascorbate peroxidase activities under sUV-B radiation compared to 1.5 times the recommended N/K levels. sUV-B significantly increased total phenolics and flavonoids in plants at the recommended and 1.5 times the recommended NPK, while flavonoids declined at 1.5 times the recommended N. This study clearly showed that NPK amendment provided maximum protection to photosynthetic assimilation of potato plants under sUV-B radiation, activating the antioxidative defense system as well as flavonoids. NPK at 1.5 times the recommended dose, however, did not cause any additional benefit to

photosynthetic carbon fixation; hence the recommended dose of NPK is found to be the best suited dose of fertilizer under ambient as well as sUV-B regime.

**Keywords** sUV-B radiation · NPK levels · Photosynthesis · Antioxidant

## Introduction

Stratospheric ozone ( $\text{O}_3$ ) layer depletion increases solar ultraviolet-B (UV-B) radiation (280–315 nm) on the Earth's surface. Natural variations in UV-B radiation are observed frequently at the ground level. These variations are produced by the natural latitudinal gradient in total atmospheric  $\text{O}_3$  column, prevailing thickness, solar angles at different latitudes, elevation above sea level and optical amplification effect. Thus, the amount of UV-B radiation reaching the tropical latitudes is higher than the temperate regions (Surabhi et al. 2009). Sahoo et al. (2005) observed significant declining trend of total ozone column (TOC) over numerous stations lying in the northern part of India, suggesting potential vulnerability of plants to UV-B under field conditions.

Plants absorb photons to power the photosynthesis; thus they are inevitably exposed to UV-B (Paul and Gwynn-Jones 2003). Investigations of the modified effects of UV-B radiation on plants have been carried out in growth chambers, greenhouse and in the field. UV-B affected morphological, physiological and metabolic responses including altered plant growth, reduced yield, damage to photosystem II and decrease in chlorophyll content (Germ et al. 2005). Plants can produce a wide range of UV-B absorbing compounds to protect themselves from UV-B radiation damage, and these substances tend to accumulate in the leaves of higher plants (Dolzhenko et al. 2010). Also, plants are able

Communicated by Z. Miszalski.

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

to activate antioxidant enzymes which scavenge free radicals, thus offering protection to lipids, proteins and nucleic acids (reviewed by Agrawal et al. 2009). UV-B radiation can impair all major processes of photosynthesis including photochemical reactions in thylakoid membranes, enzymatic processes in the Calvin cycle and stomatal limitations to CO<sub>2</sub> diffusion (Allen et al. 1998).

Under realistic field conditions, plants are exposed to multiple environmental factors such as drought, temperature, heavy metals and nutrients, which may modify the plant's response to UV-B (Prasad et al. 2005; Singh et al. 2011a; Agrawal and Rathore 2007). Phosphorus deficiency in soil led soybean plants to be less sensitive to UV-B than plants grown at optimum P levels due to accumulation of secondary compounds and leaf thickening (Murali and Teramura 1985). Study of the response of potato to UV-B in a growth chamber reported increased flavonoid content, slight reduction in chlorophyll and protein contents and activation in antioxidant defense (Santos et al. 2004). Singh et al. (2011b) observed that potato plants grown at higher than recommended NPK utilized the resources in promoting growth, whereas recommended NPK maintained the balance between growth and yield even under UV-B stress.

Since the major nutrients (NPK) play important role in the development of plants by modifying various physiological and metabolic activities, the present study investigates the interactive influence of UV-B radiation and varying levels of NPK on an important vegetable crop, *Solanum tuberosum* L. var Kufri Badshah (Potato) grown under ambient and supplemental UV-B<sub>BE</sub> (ambient + 7.2 kJ m<sup>-2</sup> d<sup>-1</sup> biologically effective UV-B above ambient UV-B level) under natural field conditions to determine the supplemental UV-B effects on (1) chlorophyll fluorescence, CO<sub>2</sub> assimilation and gas exchange; (2) enzymatic and non-enzymatic antioxidative potential and metabolites; (3) starch partitioning to understand the mechanism of action involved in growth and yield reductions.

This study is a part of an experiment conducted by Singh et al. (2011b), in which the growth and yield responses of test plant to UV-B and different nutrient regimes were reported. The present study aimed to assess the physiological and biochemical mechanism in test plants under sUV-B at different NPK levels and to correlate these mechanisms with growth and yield.

## Materials and methods

### Experiment site and plant material

The field experiment was conducted from November 2006 to February 2007 at the Botanical Garden of Banaras Hindu

University, Varanasi, Uttar Pradesh (25°81'N, 83°1'E; 76 m above sea level), situated in the mid Gangetic plain of India. During the experiment, the mean temperature ranged from 13.6 to 28.4 °C, mean relative humidity from 38.8 to 72.5 % and total rainfall was 114.3 mm. Photosynthetically active radiation (PAR) averaged 1,000 μmol m<sup>-2</sup> s<sup>-1</sup> at midday.

Potato (*S. tuberosum* L.) var. Kufri Badshah was selected as the test plant and it was developed and released by the Central Power Research Institute (CPRI) and All India Co-ordinated Pulses Improvement Project (AICPIP).

### Experimental design and NPK application

The experimental design was a split plot with UV-B treatment as main plot and NPK treatment as subplots randomized within the whole plot, with three replicates per treatment. The experiment had three factors: (1) UV-B treatment, (2) four NPK amendments and (3) plant age. The four NPK treatments were: the recommended dose of NPK (F<sub>0</sub>), 1.5 times the recommended dose of NPK (F<sub>1</sub>), 1.5 times the recommended dose of N (F<sub>2</sub>) and 1.5 times the recommended dose of K (F<sub>3</sub>). For convenience, control plants grown at ambient UV-B were designated F<sub>0</sub>C, F<sub>1</sub>C, F<sub>2</sub>C and F<sub>3</sub>C, and the corresponding UV-B treated plants as F<sub>0</sub>T, F<sub>1</sub>T, F<sub>2</sub>T and F<sub>3</sub>T. The recommended doses of NPK were 150, 100 and 30 kg ha<sup>-1</sup>, respectively. A half dose of N and full doses of P and K were given as a basal dressing before sowing tubers, and a further half dose of N was given as a top dressing after 7 of germination.

Before sowing the tubers, the soil was sampled for estimating NPK levels and then the deficits from the required level were added. The actual and deficit amounts added to reach the required concentration of NPK in the soil are given in Table 1. The actual concentrations of NPK after addition of NPK are given in Table 1. Potato tubers were sown in rows in 24 plots of 1 × 1 m each. For each treatment three replicate plots were maintained. Potato tubers were sown on ridges, with 45 cm spacing between ridges. Plants were thinned to maintain a distance of 20 cm between plants in each row for uniformity in growth. Plants were watered uniformly.

### sUV-B treatment

sUV-B was artificially provided with Q panel UV-B 313 40 W fluorescent lamps (Q Panel Inc., Cleveland, OH, USA). Three lamps (120 cm long) per bank were fitted 30 cm apart on a steel frame and suspended perpendicular to the planted rows of each plot. The lamps were covered with either a 0.13 mm cellulose diacetate filter (transmission down to 280 nm) for supplemental UV-B (sUV-B) or a 0.13 mm polyester filter (absorbed radiation below

**Table 1** Recommended, deficit and actual amount of NPK added for attaining the required NPK level in the soil

	Recommended NPK (kg ha <sup>-1</sup> )	1.5 × recommended NPK (kg ha <sup>-1</sup> )	1.5 × recommended N (kg ha <sup>-1</sup> )	1.5 × recommended K (kg ha <sup>-1</sup> )
Recommended amount	150:100:30	225:150:45	225	45
Deficit amount added	40:40:9	115:90:24	115	24
Actual concentrations of NPK	144.6:98.6:28.9	223.7:148.9:43.7	222.5	43.1

320 nm) for the control. The control plants thus received only ambient levels of UV-B. Cellulose diacetate removes UV-C wavelengths emitted by lamps, which otherwise are not a component of natural sunlight. Lamps in frames were adjusted weekly to a distance of 50 cm above the plants to provide mean supplemental UV-B radiation of 7.2 kJ m<sup>-2</sup> d<sup>-1</sup> (unweighted) to plant apices for 3 h daily over the middle of the 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 UVBBE values by comparison with a Spectro Power meter (Sciencetech, Boulder, CO, USA). Plants under polyester filtered lamps received only ambient UV-B (9.6 kJ m<sup>-2</sup> d<sup>-1</sup>) at the summer solstice, weighted against the generalized plant response action spectrum of Caldwell (1971). The plants beneath the cellulose diacetate film received ambient + sUV-B (ambient + 7.2 kJ m<sup>-2</sup> d<sup>-1</sup>) that mimicked a 20 % reduction in stratospheric ozone at Varanasi during clear sky conditions (Green et al. 1980), normalized at 300 nm, zero albedo and 1.0 scatter.

#### Physiological parameters

Photosynthetic rate ( $P_s$ ) and stomatal conductance ( $g_s$ ) were measured using portable photosynthetic system (Model LI-6200, LI-COR, Lincoln, NE) at 40 and 60 days after germination (DAG). The measurements were made on the third fully expanded leaf from the top of plants on cloud free days between 08.00 and 10.00 h local time on three randomly selected plants in each plot. During the measurements, photosynthetically active radiation ranged between 1,100 and 1,200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Model LI-6200, LI-COR, Lincoln, NE). The system was calibrated using a known CO<sub>2</sub> source of 509 ppm concentration. During measurement of photosynthesis, the mean temperature ranged between 15.5 and 16.2 °C, relative humidity ranged between 45.3 and 47.8 %. Water use efficiency (WUE) was calculated as a ratio of photosynthesis to transpiration.

Chlorophyll fluorescence characteristics such as initial fluorescence ( $F_0$ ), maximum fluorescence ( $F_m$ ), variable fluorescence ( $F_v = F_m - F_0$ ) and  $F_v/F_m$  ratio were measured for the different treatments. Chlorophyll fluorescence

was determined between 10.00 and 11.00 h using portable plant efficiency analyzer (PEA, Hansatech Instruments Ltd., UK) on the same leaves where  $P_s$  was measured. Leaf clips for dark adaptation were placed on the adaxial side of the leaves for 30 min before measurement at saturating flash of 3,000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

#### Estimation of pigments, antioxidants and metabolites

Random samplings of leaves were done in triplicate at 40 and 80 DAG from each plot and were cut and stored at -20 °C before analyses of photosynthetic pigments, antioxidants and metabolites. There were nine replicates of each treatment.

#### Pigments

For pigment determination, 500 mg of leaf samples was homogenized in 10 ml of 80 % acetone and centrifuged at 6,000×g for 15 min. The optical densities (O.D.) of supernatant were measured at 645 and 663 nm for chlorophylls on a UV-VIS spectrophotometer (Systronics Model 119, India). The amounts of chlorophyll a and b were calculated by using the formulae given by Maclachlan and Zalik (1963). For flavonoids determination, 0.1 g of leaf discs of 100 mm<sup>2</sup> was taken and extracted in 10 ml of acidified methanol (79:20:1 v/v, methanol, water, HCl) according to the procedure of Mirecki and Teramura (1984). Extract absorbance at 305 nm measured on a UV-VIS spectrophotometer (Model 119, Systronics, India) was used as a measure of flavonoid content.

#### Lipid peroxidation

Lipid peroxidation (LPO) in the leaf tissues was determined in terms of malondialdehyde (MDA, a product of LPO) content by thiochloroacetic 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). Ascorbate peroxidase (APX) activity was measured spectrophotometrically using the methods described by Nakano and Asada (1987). 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 done 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 color 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  $\text{Na}_2\text{CO}_3$  (Bray and Thorpe 1954). Determination of proline was performed by ninhydrin test as described by Bates et al. (1973).

#### Starch content

For starch measurement, three plants were harvested at 60 DAG from each replicate plot of each treatment. Roots were washed properly under running tap water. After separating different plant parts, i.e., leaf, stem, stolon and tuber. The materials were oven dried at 90 °C in a hot air oven till a constant weight is achieved. The dried material was ground in a stainless steel grinder and passed through a sieve of 1 mm. The dried material is used for measurement of starch using the method of Dubois et al. (1956). Starch partitioning was calculated by dividing the starch of an organ by the total starch, multiplied by 100.

#### NPK content

For N, P and K analysis, from each replicate treatment plot (3), leaves (3) from three plants were harvested at 60 DAG. Leaves collected from each plant were treated as one replicate. For determination of P and K, 0.1 g of the powdered sample was digested in a mixture of  $\text{HClO}_4$ ,  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$  (5:1:1) by following the method of Allen et al. (1986). The digested samples were filtered through Whatman no. 42 filter paper and the volume was maintained at 25 ml with distilled water. The concentration of K in the filtered solution was determined with the help of an atomic absorption spectrophotometer (Model 2380, Perkin Elmer, USA), and of P by the method given by Jackson (1958). Total N was quantified by micro-kjeldahl technique through Gerhardt Automatic N Analyzer (Model KB8S, Germany).

#### Statistical analysis of data

The statistical significance of the data for biochemical and physiological parameters was tested through three-way

analysis of variance (ANOVA) tests to examine the individual and combined effects of age, NPK amendment and UV-B treatment, and two-way ANOVA tests to examine the individual and combined effects of NPK amendment and UV-B treatment on leaf starch content. Significantly different means between control and its respective sUV-B treated plants were calculated using the ‘Student’s *t* test’. All the statistical tests were performed using SPSS software (SPSS Inc., version 16.0).

## Results and discussion

Significant negative impacts of sUV-B on different measured parameters of potato plants were observed under varying NPK levels.

#### Stress indicator

Three-way ANOVA showed that the individual factors of age (*A*), NPK (*N*) and UV-B treatment (*T*) and their interactions significantly affected MDA and chlorophyll content,  $g_s$  and WUE, while  $P_s$  varied with all the individual factors and their interactions except  $A \times T$  and  $A \times N \times T$  interactions (Table 2).

Malondialdehyde (MDA), an indicator of lipid peroxidation, increased significantly due to sUV-B treatment under all NPK levels and at all ages (Fig. 1). Lipid peroxidation, representing the damage to cellular membranes, has been reported to accelerate in plants under UV-B (Panagopoulos et al. 1990). Percent increments in MDA content in  $F_0T$ ,  $F_1T$ ,  $F_2T$  and  $F_3T$  plants were 20.6, 30.2, 58.7 and 86.5, respectively, at 80 DAG compared to their corresponding controls (Fig. 1). Similarly, Kumari et al. (2009) also reported 194.5 % increment in MDA content of *Abelmoschus esculentus* under sUV-B radiation of  $1.8 \text{ kJ m}^{-2} \text{ d}^{-1}$  over the ambient dose.  $F_2T$  and  $F_3T$  plants grown under no additional P showed maximum MDA content, suggesting that plants under low P recover phosphate from lipids and nucleic acids (Schachtman and Shin 2007). Ticconi and Abel (2004) showed that under low P availability, lipid composition shifts from phospholipids to more galacto- and sulfolipids. Phospholipid imparts rigidity to cell membrane and its loss or conversion to some other form causes excessive membrane fluidity or lipid peroxidation, which may enhance cell permeability and cell death during stress (Huang 2010). Comparatively higher P content observed at 60 DAG in  $F_2T$  may have helped to maintain the membrane integrity and function as evidenced by lower MDA content at 40 DAG, while at 80 DAG a sharp decline in N content in leaves may have led to higher MDA content (Fig. 1). Huang et al. (2004)

**Table 2** *F*-ratios and levels of significance of three-way ANOVA test for different parameters of potato

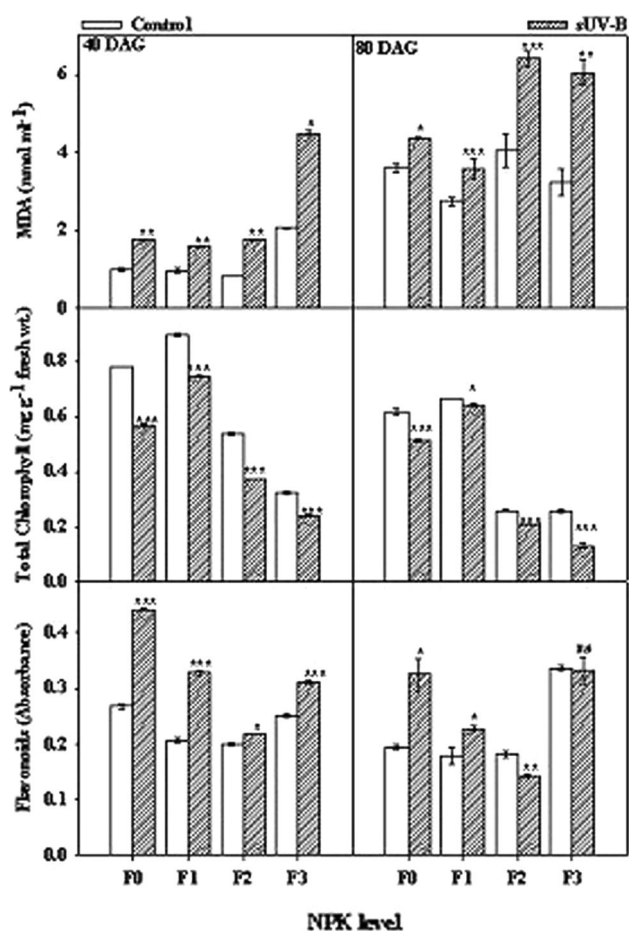
	Age (A)	NPK (N)	Treatment (T)	A × T	A × N	N × T	A × N × T	
Photosynthesis	318.9***	750.2***	272.1***	0.12 <sup>ns</sup>	2.9*	7.8***	0.93 <sup>ns</sup>	
Stomatal conductance	2,458.5***	2,033.1***	488.4***	22.3***	42.8***	30.3***	13.4***	
Water use efficiency	343.3***	205.0***	30.4***	44.6***	3.4*	5.5**	13.3***	
Chlorophyll	2,557.5***	6,899.7***	1,655.8***	183.7***	112.7***	28.6***	54.7***	
Flavonoids	50.5***	123.6***	140.9***	30.9***	36.2***	43.7***	0.37 <sup>ns</sup>	
MDA content	728.2***	68.8***	252.1***	7.6**	37.6***	24.4***	3.1*	
SOD	4,019.0***	241.4***	1,436.7***	181.5***	66.8***	124.7***	79.3***	
POX	174.5***	421.3***	65.5***	1.4 <sup>ns</sup>	21.7***	79.1***	135.1***	
APX	341.2***	357.9***	0.05 <sup>ns</sup>	103.1***	289.8***	285.6***	258.9***	
Ascorbic acid	9.4**	59.8***	21.3***	13.5***	1.6 <sup>ns</sup>	28.4***	33.5***	
Phenol	3,837.5***	4,414.1***	384.7***	64.9***	123.7***	418.3***	195.8***	
Protein	826.5***	38.2***	259.6***	4.9*	44.0***	10.4***	33.3***	
Proline	5,192.9***	575.5***	2,652.3***	394.3***	449.3***	122.3***	62.7***	
Initial fluorescence	426.7***	5.2***	7.5***	0.15 <sup>ns</sup>	17.8***	20.0***	0.14 <sup>ns</sup>	
Level of significance: <i>ns</i> not significant	$F_m$	232.4***	251.9***	88.1***	0.23 <sup>ns</sup>	32.7***	22.7***	0.18 <sup>ns</sup>
	$F_v$	510.6***	225.5***	70.8***	0.11 <sup>ns</sup>	42.6***	28.0***	0.30 <sup>ns</sup>
* $p \leq 0.05$ , ** $p \leq 0.01$ , *** $p \leq 0.001$	$F_v/F_m$	1,053.1***	1,605.1***	22.3***	944.8***	49.6***	144.4***	58.9***

reported overaccumulation of MDA in N-deficient rice plants.

Increase in lipid peroxidation enhanced the photodisintegration of thylakoid membrane of chloroplast leading to the loss of chlorophyll under UV-B treatment at all NPK levels. Total chlorophyll content decreased in F<sub>0</sub>T (27.1 and 16.5 %), F<sub>1</sub>T (16.7 and 3.3 %), F<sub>2</sub>T (30.9 and 19.1 %) and F<sub>3</sub>T (25.3 and 49.5 %) plants compared to their respective controls at both ages (40 and 80 DAG) of observations (Fig. 1). Teramura (1983) attributed reduction in chlorophyll a and b to inhibition of biosynthesis or degradation of chlorophyll and their precursors under enhanced UV-B. sUV-B is known to cause photooxidation of newly synthesized pigments (Strid and Porra 1992) and damage to the photosystem proteins, which may modify the assembly of chlorophyll during chloroplast development. Chlorophyll content (absolute term) was maximum in F<sub>1</sub>C and minimum in F<sub>3</sub>C among the controls. Sosik and Mitchell (1991) also reported that increase in N content in soil led to increments in chlorophyll a and b in *Dunaliella tertiolecta*. But the loss of pigment relative to the respective controls under sUV-B was maximum in F<sub>2</sub>T at 40 DAG and F<sub>3</sub>T at 80 DAG, and minimum in F<sub>1</sub>T at both ages. As chlorophyll molecules contain a majority of foliar N (Peterson et al. 1993), the higher than the recommended dose of N (F<sub>1</sub>) may have helped in retaining higher chlorophyll compared to the recommended NPK even under UV-B stress. Higher phosphorus and potassium availability

has been shown to increase chlorophyll content in maize (Jiang et al. 2007) and marigold (Pal and Ghosh 2010). Availability of higher than recommended level of N in F<sub>2</sub>C could not help the plants in maintaining chlorophyll because of low actual leaf N concentration. Leaf N content in F<sub>2</sub>T plants was lowest among all the treatments. Crops that are grown in soil low in K show low total N uptake (Jones et al. 2011).

Photosynthesis rate did not differ significantly between control (F<sub>0</sub>C) and UV-B treated (F<sub>0</sub>T) plants under the recommended NPK (Table 3), however, decreased in F<sub>1</sub>T, F<sub>2</sub>T and F<sub>3</sub>T compared to F<sub>1</sub>C, F<sub>2</sub>C and F<sub>3</sub>C, respectively (Table 3). The trend of reduction was F<sub>3</sub>T = F<sub>2</sub>T > F<sub>1</sub>T. This suggests that the photosynthetic apparatus of potato adapts and readily tolerates ambient and supplemental UV-B when supplied with the recommended NPK. Significant reductions in stomatal conductance were, however, observed under sUV-B treatment at all NPK amendments, the maximum decline being in F<sub>2</sub>T (31.0 and 44.2 %) and minimum in F<sub>1</sub>T (7.6 and 13.9 %) plants at 40 and 60 DAG, respectively, compared to their controls (Table 3). Reduction in CO<sub>2</sub> assimilation is associated with stomatal closure, which simultaneously reduces water loss (Peñarajas et al. 2004). The absence of reduction in  $P_s$  with a reduction in  $g_s$  in F<sub>0</sub>T compared to F<sub>0</sub>C suggests that there may have been biochemical compensation within the photosynthetic apparatus. Nogués et al. (1998) also recorded that photosynthesis rate did not correspond directly to



**Fig. 1** Effects of sUV-B treatment on MDA, total chlorophyll and flavonoid content of potato plants under varying NPK levels at 40 and 80 DAG. 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. F<sub>0</sub>: recommended dose of NPK; F<sub>1</sub>: 1.5 times the recommended dose of NPK; F<sub>2</sub>: 1.5 times the recommended dose of N; F<sub>3</sub>: 1.5 times the recommended dose of K

stomatal conductance under UV-B in pea. In C<sub>3</sub> plants, when stomata close in response to UV-B and CO<sub>2</sub> assimilation is reduced, the photosynthetic reduction of O<sub>2</sub> via photorespiration increases and serves as a sink for excess excitation in the photosynthetic apparatus (Cornic and Briantais 1991). However, increase in the photorespiratory reduction of O<sub>2</sub> is not sufficient to dissipate the excess excitation energy in PSII antennae, and consequently increased dissipation of this energy as heat occurs to minimize photodamage to the PSII reaction centers (Baker 1994).

The performance of the photosynthetic system depends on many factors including the quenching efficiency of light during the photochemical reactions. Measurement of the chlorophyll fluorescence is a quick, precise and non-destructive technique, widely used in investigating damage/repair caused in the photosynthetic plant system by

various types of stresses (Govindjee 1995).  $P_s$  was more affected in F<sub>2</sub>T and F<sub>3</sub>T at both ages, but  $F_v/F_m$  remained unaffected at 40 DAG, reflecting sensitivity of dark reaction in these treatments (Table 3). At 60 DAG, however, maximum reduction of  $F_v/F_m$  in F<sub>3</sub>T plants directly corresponded with decrease in  $P_s$  (Table 3). On the other hand, significant decrease in gas exchange parameters in F<sub>2</sub>T under sUV-B radiation with insignificant (40 DAG) or relatively small decrease (at 60 DAG) in  $F_v/F_m$  suggests that the demands for reductants and ATP have decreased dramatically (Demmig-Adams et al. 1996), which may be a factor for the closure of PSII reaction centers. Non-functional PSII centers may act as dissipative sinks, thereby decreasing  $F_0$  (Bjoerkman and Demming 1987), which may be accounted for by the decreasing trend in F<sub>1</sub>T and F<sub>2</sub>T, although this was statistically insignificant in F<sub>2</sub>T. sUV-B radiation led to significant reductions of 11.4, 10.6 and 5.5 % in  $F_m$  values of F<sub>1</sub>T, F<sub>2</sub>T and F<sub>3</sub>T plants at 60 DAG compared to controls (Table 3). Contrasting response of F<sub>0</sub>T and F<sub>3</sub>T with respect to  $F_m$  and  $P_s$  revealed that the decrease in  $F_m$  in F<sub>0</sub>T was not related to damage, but resulted from a safe down-regulation of PSII efficiency through increases in non-radiative energy dissipation (Demmig-Adams et al. 1996). More reduction in  $g_s$  in F<sub>3</sub>T than F<sub>0</sub>T might have further increased the reduced state of quinone acceptor (Lovell and Winter 1996). An increase in reduction state of PSII may have exposed the leaves to damage, thus leading to larger decline in  $F_v/F_m$ , as was observed in F<sub>3</sub>T compared to other UV-B treatments at 60 DAG. Hikosaka et al. (2004) examined the relationship between  $F_v/F_m$  and CO<sub>2</sub> assimilation rate in photoinhibited *Chenopodium album* L. and reported that CO<sub>2</sub> assimilation rate declined in parallel with decreasing  $F_v/F_m$ . Three-way ANOVA results confirmed that interactive effects of  $A \times T$  and  $A \times N \times T$  did not significantly affect  $F_m$ ,  $F_0$  and  $F_v$  (Table 2).

WUE decreased in F<sub>1</sub>T (19.2 %) at 40 DAG, whereas it increased in F<sub>1</sub>T (16.9 %) plants at 80 DAG (Table 3). The increase in WUE despite reduction in photosynthesis can be ascribed to less reduction in  $P_s$  than transpiration (Jones 1993). Many studies have reported that sUV-B radiation induced a decrease in instantaneous WUE under growth chamber, greenhouses and field conditions (Greitner and Winner 1988). However, Greitner and Winner (1988) reported that the impact of UV-B on carbon fixation was smaller than on stomatal conductance, and therefore improvement in WUE was observed. Qaderi et al. (2007) also reported reduction in WUE (14.9 %) of *Silvialas* sp. grown under 4.2 kJ m<sup>-2</sup> d<sup>-1</sup> of UV-B. Maximum reduction in WUE was recorded in F<sub>3</sub>T plants grown at low N and low P. Virgona and Farquhar (1996) also observed low WUE under low N-supply, while higher WUE was reported with high N in *Ipomoea batatas* (Kelm et al. 2001).

**Table 3** Effects of sUV-B on photosynthesis, stomatal conductance, water use efficiency, initial fluorescence, maximum fluorescence, variable fluorescence and  $F_v/F_m$  of potato plants at 40 and 60 DAG under varying soil NPK levels

Age (DAG)	NPK level	sUV-B treatment	Photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	Stomatal conductance ( $\text{mol m}^{-2} \text{ s}^{-1}$ )	Water use efficiency ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )	Initial fluorescence	Maximum fluorescence	Variable fluorescence	$F_v/F_m$
40	F <sub>0</sub>	C	14.32 ± 0.698	0.985 ± 0.002	3.23 ± 0.029	432.7 ± 23.4	2,749 ± 4.6	1,779.7 ± 25.7	0.797 ± 0.010
		T	13.5 ± 0.378 <sup>ns</sup>	0.88 ± 0.006**	2.92 ± 0.095 <sup>ns</sup>	454.3 ± 22.2	2,234 ± 4.0***	2,316.3 ± 25.8**	0.843 ± 0.009 <sup>ns</sup>
	F <sub>1</sub>	C	16.26 ± 0.029	1.3 ± 0.012	3.69 ± 0.008	501.8 ± 0.95	2,452 ± 0.75	1,775 ± 4.6	0.815 ± 0.0003
		T	14.57 ± 0.173***	1.22 ± 0.003***	2.98 ± 0.008*	403.0 ± 1.73***	2,178 ± 6.4***	1,950.5 ± 0.20***	0.795 ± 0.0003***
	F <sub>2</sub>	C	12.85 ± 0.026	0.74 ± 0.003	4.14 ± 0.010	399.3 ± 13.2	2,534.2 ± 7.6	1,873.7 ± 16.9	0.824 ± 0.0061
		T	10.14 ± 0.015***	0.51 ± 0.003***	4.08 ± 0.069 <sup>ns</sup>	381.7 ± 5.8 <sup>ns</sup>	2,273 ± 4.6**	2,152.5 ± 12.0**	0.849 ± 0.003 <sup>ns</sup>
60	F <sub>3</sub>	C	9.15 ± 0.017	0.56 ± 0.003	4.15 ± 0.012	440.3 ± 7.86	2,257 ± 3.4	1,663.1 ± 7.5	0.781 ± 0.002
		T	7.22 ± 0.058**	0.46 ± 0.013 <sup>ns</sup>	3.62 ± 0.02**	465.9 ± 4.0 <sup>ns</sup>	2,256.9 ± 4.6**	1,816.6 ± 11.2*	0.805 ± 0.004 <sup>ns</sup>
	F <sub>0</sub>	C	12.29 ± 0.71	0.74 ± 0.05	2.50 ± 0.11	587.7 ± 12.5	2,648 ± 105.3	2,060.7 ± 114.7	0.784 ± 0.009
		T	10.83 ± 1.52 <sup>ns</sup>	0.62 ± 0.03*	2.58 ± 0.14 <sup>ns</sup>	626.7 ± 27.9 <sup>ns</sup>	2,114 ± 85 <sup>ns</sup>	1,487.3 ± 68.9 <sup>ns</sup>	0.691 ± 0.009*
	F <sub>1</sub>	C	14.24 ± 1.38	0.86 ± 0.04	2.42 ± 0.14	603 ± 2.9	2,306 ± 11.6	1,433 ± 43.4	0.723 ± 0.004
		T	12.55 ± 1.53*	0.74 ± 0.02*	2.83 ± 0.15**	512.3 ± 7.3**	2,044 ± 31.8*	1,131 ± 16.5 <sup>ns</sup>	0.571 ± 0.002**
F <sub>2</sub>	C	10.74 ± 0.71	0.46 ± 0.02	3.43 ± 0.19	594 ± 10.7	1,945 ± 23	1,668 ± 163.3	0.636 ± 0.005	
	T	8.25 ± 0.44***	0.26 ± 0.01**	3.37 ± 0.16 <sup>ns</sup>	572.7 ± 14.1 <sup>ns</sup>	1,739.3 ± 16.9*	1,588 ± 57*	0.526 ± 0.004**	
F <sub>3</sub>	C	7.60 ± 0.46	0.24 ± 0.01	3.55 ± 0.19	513.3 ± 1.8	1,959.3 ± 15.5	1,916 ± 11.8	0.523 ± 0.003	
	T	5.84 ± 0.25**	0.20 ± 0.01***	3.27 ± 0.18**	537.7 ± 5.8 <sup>ns</sup>	1,851.7 ± 9.1*	1,421 ± 16**	0.359 ± 0.021*	

Values are mean ± 1SE

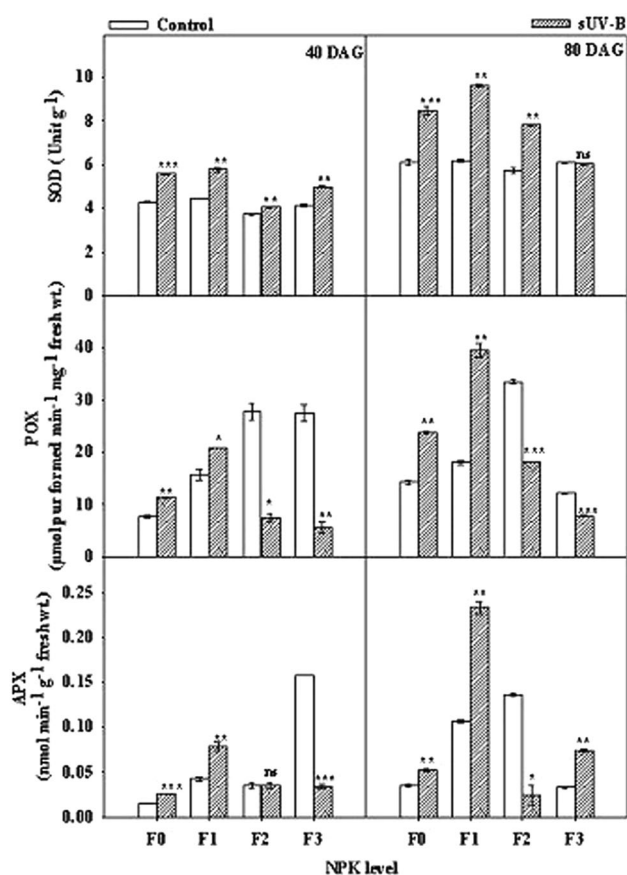
Level of significance between control and sUV-B treated potato plants

<sup>ns</sup> not significant, F<sub>0</sub> recommended dose of NPK, F<sub>1</sub> 1.5 times the recommended dose of NPK, F<sub>2</sub> 1.5 times the recommended dose of NPK, F<sub>3</sub> 1.5 times the recommended dose of K\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

## Antioxidant and biochemical responses

Flavonoids varied significantly with age, NPK and UV-B treatment and their interactions, except  $A \times N \times T$  interaction (Table 2). The UV-B screening ability of flavonoids is known to reduce the degree of damage to the photosynthetic tissue. An increase in flavonoid content in response to low availability of N and P has been previously reported (Müller et al. 2007). The percent change in flavonoids, however, was highest in F<sub>0</sub>T plants compared to the respective controls at both ages of observation (Fig. 1). Flavonoid accumulation was maximum in F<sub>3</sub>C plants at 80 DAG and F<sub>0</sub>C at 40 DAG. Despite the maximum accumulation of flavonoids in F<sub>3</sub>T plants at 80 DAG, the negative effects of UV-B on the photosynthetic apparatus could not be prevented; however, in F<sub>0</sub>T, maximum protection to photosynthetic machinery was observed. N, P and K in combination induced flavonoid synthesis under sUV-B radiation in F<sub>0</sub>T and F<sub>1</sub>T plants at both the ages (Fig. 1). At low leaf N concentration (F<sub>2</sub>), as a consequence of UV-B exposure, upregulation of secondary metabolism was lower than at the recommended (F<sub>0</sub>), 1.5 times the recommended NPK (F<sub>1</sub>) or higher K (F<sub>3</sub>).

SOD, POX and APX activities significantly varied with age, NPK, UV-B treatments and their interactions except POX, which did not vary with  $A \times T$  (Table 2). UV-B exposure enhances the generation of reactive oxygen species, and the plants metabolize them by invoking the antioxidant defense system. Transcripts of key enzymes of antioxidative enzyme systems are induced by UV-B (Willekens et al. 1994). sUV-B led to increments in SOD activities in F<sub>0</sub>T (29.7 and 38.4 %), F<sub>1</sub>T (29.5 and 55.6 %) and F<sub>3</sub>T (8.5 and 36.9 %) plants with maximum increment under 1.5 times NPK (F<sub>1</sub>T) at both the ages of observations (Fig. 2). Singh et al. (2010) also observed increase in SOD activity in radish plants under same UV-B exposure level. SOD activity decreased in *Coffea arabica* grown without N as well as at higher N level, while it was increased at the intermediate level (Ramalho et al. 1998). POX activity increased in F<sub>0</sub>T (46.1 and 65.8 %) and F<sub>1</sub>T (32.8 and 119.5 %), but decreased in F<sub>2</sub>T (73.1 and 45.6 %) and F<sub>3</sub>T (79.4 and 35.9 %) plants under sUV-B radiation compared to their respective controls (Fig. 2). However, Singh et al. (2010) observed an increment in POX activity in radish under sUV-B irradiation grown in field conditions. Singh et al. (2012) also observed reductions in POX activities in radish plants grown at the 1.5 times the recommended N/K and exposed to the same UV-B level. F<sub>2</sub>T and F<sub>3</sub>T plants showed lesser APX and POX activities (Fig. 2); therefore the conversion of produced H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O was not efficiently performed as is evident from higher MDA content in the same treatments. UV-B radiation, however, increased APX activity in *A. thaliana* (Rao et al. 1996) and *Acer mono maxim* (Yao and Liu 2006). Higher increase in SOD in F<sub>0</sub>T and F<sub>1</sub>T led to



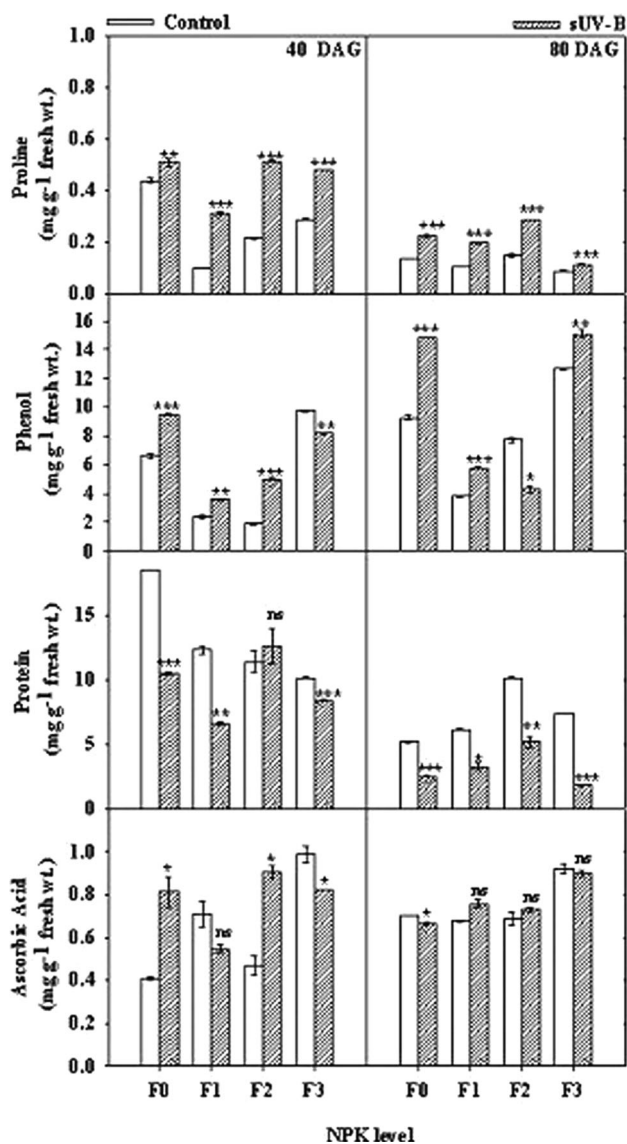
**Fig. 2** Effects of sUV-B treatment on superoxide dismutase (SOD), ascorbate peroxidase (APX) and peroxidase (POX) activities of potato plants under varying NPK levels at 40 and 80 DAG. Values are mean  $\pm$  1SE. Level of significance between control and sUV-B treated plants: ns, not significant, \* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001. F<sub>0</sub>: recommended dose of NPK; F<sub>1</sub>: 1.5 times the recommended dose of NPK; F<sub>2</sub>: 1.5 times the recommended dose of N; F<sub>3</sub>: 1.5 times the recommended dose of K

lower increase in MDA content (Figs. 1, 2). POX and APX activities also increased in F<sub>0</sub>T and F<sub>1</sub>T, which may have reduced the oxidative stress, thus reducing lipid peroxidation.

Three-way ANOVA results showed that individual factors of age, NPK and UV-B treatment and their interactions significantly affected ascorbic acid, phenol, protein and proline except ascorbic acid, which did not vary with  $A \times N$  interaction (Table 2). Ascorbic acid increased by 98.7 and 93.4 % in F<sub>0</sub>T and F<sub>2</sub>T plants under sUV-B radiation at 40 DAG (Fig. 3). Agrawal and Rathore (2007), however, observed significant reduction in ascorbic acid content in wheat and mung bean plants under sUV-B radiation with less reduction under the recommended NPK treatment and also showed that the sensitive wheat plant showed more reduction than mung bean.

At both ages of observation, proline content increased significantly at all NPK levels under sUV-B radiation with maximum increments in F<sub>1</sub>T (219.4 and 91.5 %) and F<sub>2</sub>T





**Fig. 3** Effects of sUV-B treatment on proline, phenol, protein and ascorbic acid content of potato plants under varying NPK levels at 40 and 80 DAG. 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. F<sub>0</sub>: recommended dose of NPK; F<sub>1</sub>: 1.5 times the recommended dose of NPK; F<sub>2</sub>: 1.5 times the recommended dose of N; F<sub>3</sub>: 1.5 times the recommended dose of K

(137.7 and 89.8 %) at 40 and 80 DAG, respectively, compared to their controls (Fig. 3). Alia Saradhi et al. (1995) also found that plants exposed to UV-B radiation accumulate proline and suggested that it can protect plant cells against UV-B radiation-induced peroxidative processes. The trend of the present study, however, did not show a direct correlation between proline content and UV-B tolerance.

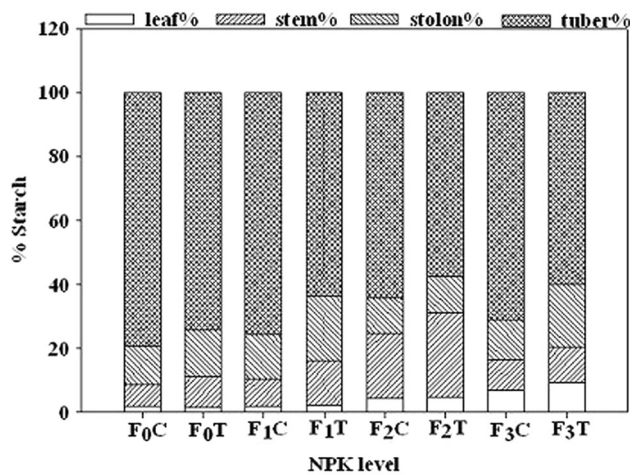
Age-wise differential response of plants for phenolic content was observed against sUV-B at varying NPK levels (Fig. 3). Total phenolics was maximum in F<sub>3</sub>C plants and,

as a consequence of UV-B treatment, increased in F<sub>0</sub>T (59.9 %), F<sub>1</sub>T (52.8 %) and F<sub>3</sub>T (19.0 %), whereas F<sub>2</sub>T (43.9 %) exhibited reduction at 80 DAG (Fig. 3). The total phenolics were highest under 1.5 times the recommended K which support Vergeer and van der Velde (1997), who claimed that if N was sufficiently present, the enhancement of phenolic production was diminished. Under sUV-B treatment, however, induction of total phenolics was higher in F<sub>0</sub>T and F<sub>1</sub>T plants, suggesting that the greater availability of photosynthates under UV-B in leaves were utilized in the production of phenolics. F<sub>2</sub>T plants also showed higher total phenolics at 40 DAG, while protein did not vary significantly under sUV-B. But at 80 DAG, both protein and phenolics declined significantly under sUV-B, which suggests a disturbed carbon partitioning between the metabolites at low leaf N concentration.

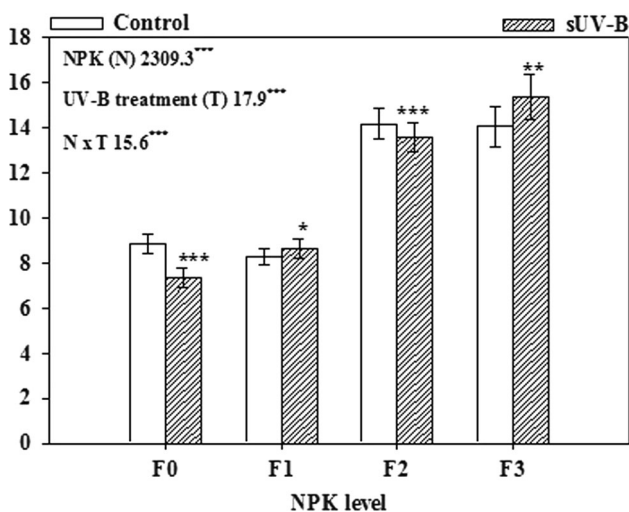
Higher protein content was observed in F<sub>0</sub>C at 40 DAG and F<sub>1</sub>C at 80 DAG; however, F<sub>0</sub>T, F<sub>1</sub>T, F<sub>2</sub>T and F<sub>3</sub>T plants showed reductions of 51.2, 46.8, 48.4 and 75.4 %, respectively, in protein content under sUV-B radiation at 80 DAG, compared to their controls (Fig. 3). UV-B radiation is known not only to cause modification or destruction of amino acid residues, but also to inactivate protein and enzymes (Prinsze et al. 1990). Decrease in protein content under enhanced UV-B stress is commonly observed (Yuichi et al. 1989). In some plants, potassium is known to substantially affect protein synthesis (Marschner 1995). However, in F<sub>3</sub>T plants under UV-B stress, minimum protein content was followed by maximum phenolics. In most of the treatments, increase in phenols was accompanied by decrease in proteins except in F<sub>3</sub>T at 40 DAG and F<sub>2</sub>T at 80 DAG, suggesting that there exists an inverse correlation between these two metabolites. As per the protein competition model of phenolic allocation, these two metabolites are inversely related because of competition for the limiting precursor phenylalanine (Jones and Hartley 1999).

#### Starch partitioning and NPK content

The trend of starch partitioning in different plant parts showed that under sUV-B, it declined in tubers at all NPK treatments, but increased in stem and stolon (Fig. 4). Except in F<sub>0</sub>T, starch partitioning in leaves increased in F<sub>1</sub>T, F<sub>2</sub>T and F<sub>3</sub>T under sUV-B (Fig. 5). This trend further suggests that UV-B stress led to unfavorable impact on translocation of photosynthates and under the recommended NPK this disturbance was minimum as partitioning was more in tuber compared to other UV-B treated plants. This has led to maintenance of higher yield in F<sub>0</sub>T plants (Singh et al. 2011b). Singh et al. (2011b) observed more translocation of biomass to tubers at the recommended NPK level under sUV-B radiation. Maximum partitioning

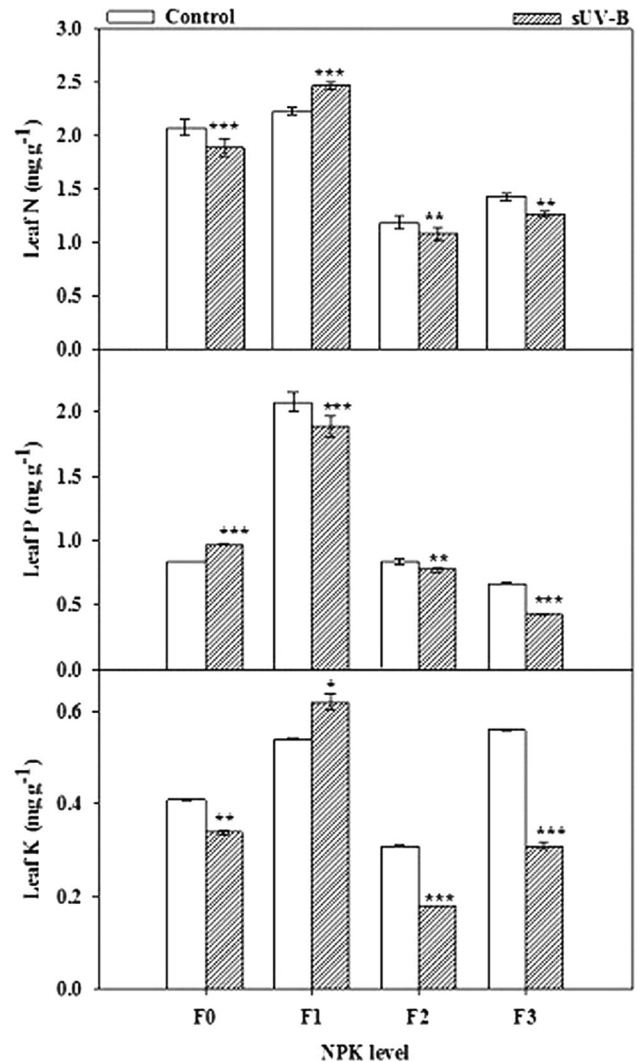


**Fig. 4** Starch (%) distribution in different parts of potato plants grown under varying NPK levels in control and sUV-B treated plants. F<sub>0</sub>: recommended dose of NPK; F<sub>1</sub>: 1.5 times the recommended dose of NPK; F<sub>2</sub>: 1.5 times the recommended dose of N; F<sub>3</sub>: 1.5 times the recommended dose of K



**Fig. 5** Effects of sUV-B treatment on leaf starch content of potato plants under varying NPK levels at 60 DAG. Values are mean  $\pm$  SE. Results of two-way ANOVA shown as NPK levels (N), UV-B treatments (T) and N  $\times$  T interaction. Level of significance between control and sUV-B treated plants: ns, not significant, \* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001. F<sub>0</sub>: recommended dose of NPK; F<sub>1</sub>: 1.5 times the recommended dose of NPK; F<sub>2</sub>: 1.5 times the recommended dose of N; F<sub>3</sub>: 1.5 times the recommended dose of K

of starch in leaves was observed in F<sub>3</sub>T (9.5 %) (Fig. 4). Plants grown under low P availability have been already found to accumulate carbohydrates in leaves (Rao et al. 1990). Though starch partitioning was reduced in tubers, partitioning to stolon increased with maximum in F<sub>1</sub>T (20.5 %) under sUV-B radiation (Fig. 4). Reduction in leaf starch partitioning in F<sub>0</sub>T (1.59 %) can be ascribed to respiratory consumption of starch, which may have helped



**Fig. 6** Effects of sUV-B treatment on leaf nitrogen (N), phosphorus (P) and potassium (K) content of potato plants under varying NPK levels at 40 DAG. Values are mean  $\pm$  SE. Level of significance between control and sUV-B treated plants: ns, not significant, \* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001. F<sub>0</sub>: recommended dose of NPK; F<sub>1</sub>: 1.5 times the recommended dose of NPK; F<sub>2</sub>: 1.5 times the recommended dose of N; F<sub>3</sub>: 1.5 times the recommended dose of K

in partitioning the maximum to tubers of F<sub>0</sub>T (74.5 %). NPK amendment and UV-B treatment significantly affected starch content in leaves (Fig. 5). Leaf starch content decreased in sUV-B treated plants compared to their controls at all NPK amendments (Fig. 5). The percent reductions in leaf starch contents were 17.2, 14.5, 12.6 and 17.6 in F<sub>0</sub>T, F<sub>1</sub>T, F<sub>2</sub>T and F<sub>3</sub>T plants, respectively compared to their controls (Fig. 5). Cellular accumulation of starch in leaves except in F<sub>0</sub>T might have inhibited photosynthesis, either through physical disruption of chloroplast or through feedback inhibition of photosynthesis due to reduced Ru-bisco activity (Stitt 1991), and thus decreased biomass production (Singh et al. 2011b). Significant reduction in

total biomass of potato was observed under sUV-B at all NPK levels at 40 DAG (Singh et al. 2011b).

Among the controls, N, P and K were highest in F<sub>1</sub>C plants grown at 1.5 times the recommended NPK suggesting a higher NPK availability at higher NPK level. Under sUV-B radiation also, foliar N and K contents significantly increased in F<sub>1</sub>T and foliar P increased in F<sub>0</sub>T plants (Fig. 6). This trend may be ascribed to reduced biomass under sUV-B, which enhanced the nutrient content per unit weight.

## Conclusion

Significant negative effects of sUV-B on physiological and biochemical characteristics of potato plants were detected; however, the degree of damage varied with different doses and combinations of N, P and K and plant age. Potato plants grown at higher than recommended NPK (F<sub>1</sub>T), N (F<sub>2</sub>T) and K (F<sub>3</sub>T) showed more sensitivity toward sUV-B than those grown at recommended NPK (F<sub>0</sub>T). Plants developed different strategies against sUV-B under varying NPK levels. The physiological and biochemical responses of the present study showed that at the recommended NPK level, photosynthetic rate was maintained at a normal rate due to an induced antioxidative defense system and UV-B absorbing, flavonoids under sUV-B, while at 1.5 times the recommended NPK growth was more pronounced due to greater nutrient availability, but the greater loss in photosynthesis did not allow sufficient translocation to underground parts, which led to higher yield reductions compared to recommended NPK under sUV-B radiation (Singh et al. 2011b). In F<sub>0</sub>T and F<sub>1</sub>T plants, recommended and 1.5 times the recommended NPK promoted a constitutive response, and upon sUV-B irradiation a proper balance was maintained between constitutive and induced protection. Better trade-off between induced defense provided by flavonoids, phenolics, antioxidants and constitutive defense by physiological adjustments and metabolites led to maximum protection to F<sub>0</sub>T plants. Foliar starch in F<sub>0</sub>T plants was effectively utilized as substrate to release energy, which was efficiently used in defense mechanism as is evident with higher stimulation of antioxidative system under recommended NPK. Application of single nutrient was ineffective in coping with UV-B stress.

**Author contribution** Suruchi Singh performed the entire experiment, finalized the data and prepared the manuscript under the guidance of Prof Madhoolika Agrawal. Prof S.B. Agrawal was involved in experimental planning, execution and even during manuscript preparation. He was part of the joint work on evaluation of UV-B impacts on plants. We also shared the facilities and did collaborative work in the department.

**Acknowledgments** We thank Head, Department of Botany, Banaras Hindu University for providing necessary laboratory facilities. The authors are also thankful to the authorities of Banaras Hindu University and Council of Scientific and Industrial Research, New Delhi, for providing financial assistance. Our special thanks go to the reviewers of this paper for their valuable suggestions and corrections.

## 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
- Alia Saradhi PP, Arora S, Prasad KVSK (1995) Proline accumulates in plants exposed to UV radiation and protects them against UV induced peroxidation. *Biochem Biophys Res Commun* 209:1–5
- 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 (1994) Light use efficiency and photoinhibition of photosynthesis in plants under environmental stress. In: Smith JAC, Griffiths H (eds) *Water deficits, plant responses from cell to community*. βios Scientific Publishers, Oxford, pp 221–235
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. *Plant Soil* 39:205–207
- Bjoerkman O, Demming B (1987) Proton yield of O<sub>2</sub> evolution and chlorophyll fluorescence at 77 k among vascular plants of diverse origins. *Planta* 170:489–504
- 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*, vol 2. Academic Press Inc, New York, p 764
- 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
- Cornic G, Briantais JM (1991) Partitioning of photosynthetic electron flow between CO<sub>2</sub> and O<sub>2</sub> reduction in a C<sub>3</sub> leaf (*Phaseolus vulgaris* L.) at different CO<sub>2</sub> concentrations and during drought stress. *Planta* 183:178–184
- Demmig-Adams B, Adams WW III, Barker DH, Logan BA, Bowling DR, Verhoeven AS (1996) Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. *Physiol Plant* 98:253–264
- Dolzhenko Y, Berteau CM, Occhipinti A, Bossi S, Maffei ME (2010) UV-B modulates the interplay between terpenoids and flavonoids in peppermint (*Mentha × Piperita* L.). *J Photochem Photobiol B* 2:67–75
- Dubois M, Gilles KA, Hamilton JK, Roberts PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28:350–356
- Fridovich I (1974) Superoxide dismutase. *Adv Enzyme* 41:35–97
- Germ M, Kreft I, Oswald J (2005) Influence of UV-B exclusion and selenium treatment on photochemical efficiency of photosystem II, yield and respiratory potential in pumpkins (*Cucurbita pepo* L.). *Plant Physiol Biochem* 43:445–448
- Govindjee (1995) Sixty-three years since Kautsky: chlorophyll a fluorescence. *Aust J Plant Physiol* 22:131–160
- Green AES, Cross KR, Smith LA (1980) Improved analytical characterization of ultraviolet skylight. *Photochem Photobiol* 31:59–65
- Greitner BC, Winner WE (1988) Increases in δ<sup>13</sup>C values of radish and soybean plants caused by ozone. *New Phytol* 108:489–494

- Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198
- Hikosaka K, Kato MC, Hirose T (2004) Photosynthetic rates and partitioning of absorbed light energy in photoinhibited leaves. Physiol Plant 121:699–708
- Huang B (2010) Cellular membrane in stress sensing and regulation of plant adaptation to abiotic stress. In: Huang B (ed) Plant environment interactions, 3rd edn. CRC Press, Taylor and Francis group, Boca Raton, pp 1–26
- 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
- Jiang HM, Yang JC, Zhang JF (2007) Effects of external phosphorus on the cell ultrastructure and the chlorophyll content of maize under cadmium and zinc stress. Environ Pollut 147(3):750–756
- Jones HG (1993) Drought tolerance and water-use efficiency. In: Smith JAC, Griffiths H (eds) Water deficits: plant responses from cell to community. BIOS Scientific Publishers, Oxford, pp 193–203
- Jones CG, Hartley SE (1999) A protein competition model of phenolic allocation. Oikos 86:27–44
- Jones C, Olson-Rutz K, Dinkins CP (2011) Nutrient uptake timing by crops. Montana State University Extension, Bozeman
- Keller T, Schwager H (1977) Air pollution and ascorbic acid. Eur J Pathol 7:338–350
- Kelm M, Brück H, Hormann M, Sattlemacher B (2001) The effect of low nitrogen supply on yield and water use efficiency of sweet potato (*Ipomoea batatas* L.). In: Horst WJ et al (eds) Plant nutrition-food security and sustainability of agro-ecosystems. Kluwer academic publishers, Netherlands, pp 402–403
- Kumari R, Singh S, Agrawal SB (2009) Combined effects of Psoralens and ultraviolet-B on growth, pigmentation and biochemical parameters of *Abelmoschus esculentus* L. Ecotoxicol Environ Saf 72:1129–1136
- Lovelock CE, Winter K (1996) Oxygen-dependent electron transport and protection from photoinhibition in leaves of tropical tree species. Planta 198:580–587
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193:265–751
- 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
- Marschner H (1995) Mineral nutrition of higher plants, II edn. Academic Press, London
- 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
- Müller R, Morani M, Jarmer H, Nilsson L, Nilsen TH (2007) Genome wide analysis of the *Arabidopsis* leaf transcriptome reveals interaction of phosphate and sugar metabolism. Plant Physiol 143:156–171
- Murali NS, Teramura AH (1985) Effects of ultraviolet-B irradiance on soybean VI. Biomass and concentration and uptake of nutrient at varying P supply. J Plant Nutr 8:177–192
- 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
- Nogués S, Allen D, Morrison J, Baker N (1998) Ultraviolet-B radiation effects on water relations, leaf development, and photosynthesis in droughted pea plants. Plant Physiol 117:173–181
- Pal P, Ghosh P (2010) Effect of different sources and levels of potassium on growth, flowering and yield of African marigold (*Tagetes erecta* Linn.) cv. 'Siracole'. Indian J Nat Prod Resour 1(3):371–375
- Panagopoulos I, Bornman JF, Bjorn LO (1990) Effects of ultraviolet radiation and visible light on growth, fluorescence induction, ultra weak luminescence and peroxidase activity in sugar beet plants. J Photochem Photobiol B: Biol 8:73–87
- Paul ND, Gwynn-Jones D (2003) Ecological roles of solar UV radiation towards an integrated approach. Trends Ecol Evol 18:48–55
- Peña-rojas K, Aranda X, Fleck I (2004) Stomatal limitation to CO<sub>2</sub> assimilation and down-regulation of photosynthesis in *Quercus ilex* resprouts in response to slowly imposed drought. Tree Physiol 24:813–822
- Peterson TA, Blackmer TM, Francis DD, Schepers JS (1993) Using a chlorophyll meter to improve N management. Nebguide G93-1171A. Cooperative Extension Services, University of Nebraska, Lincoln
- Prasad SM, Dwivedi R, Zeeshan M (2005) Growth, photosynthetic electron transport, and antioxidant responses of young soybean seedlings to simultaneous exposure of nickel and UV-B stress. Photosynthetica 43:177–185
- Prinsze C, Bubbleman TMAR, Stevenink JV (1990) Protein damage induced by small amounts of photodynamically generated singlet oxygen or hydroxyl radicals. Biochim Biophys Acta 1038:152–157
- Qaderi MM, Reid DM, Yeung EC (2007) Morphological and physiological responses of canola (*Brassica napus*) siliques and seeds to UVB and CO<sub>2</sub> under controlled environment conditions. Environ Exp Bot 60:428–437
- Ramallo JC, Campos PS, Teixeira M, Nunes MA (1998) Nitrogen dependent changes in antioxidant system and in fatty acid composition of chloroplast membranes from *Coffea arabica* L. plants submitted to high irradiance. Plant Sci 135:115–124
- Rao IM, Fredeen AL, Terry N (1990) Leaf phosphates status, photosynthesis and carbon partitioning in sugar beet. III. Diurnal changes in carbon partitioning and carbon export. Plant Physiol 92:29–36
- 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
- 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
- Santos I, Fidalgo F, Almeida JM, Salema J (2004) Biochemical and ultrastructural changes in leaves of potato plants grown under supplementary UV-B radiation. Plant Sci 167(4):925–935
- Schachtman DP, Shin R (2007) Nutrient sensing and signaling: NPKS. Ann Rev Plant Biol 58:47–69
- Singh A, Sarkar A, Singh S, Agrawal SB (2010) Investigation of supplemental ultraviolet-B-induced changes in antioxidative defense system and leaf proteome in radish (*Raphanus sativus* L. cv. Truthful): an insight to plant response under high oxidative stress. Protoplasma 245:75–83
- Singh S, Kumari R, Agrawal M, Agrawal SB (2011a) Growth, yield and tuber quality of *Solanum tuberosum* L. under supplemental ultraviolet-B radiation at different NPK levels. Plant Biol 13(3):508–516
- Singh S, Kumari R, Agrawal M, Agrawal SB (2011b) Modification in growth, biomass and yield of radish under supplemental UV-B at different NPK levels. Ecotoxicol Environ Saf 74(4):897–903
- Singh S, Kumari R, Agrawal M, Agrawal SB (2012) Differential response of radish plants to supplemental ultraviolet-B radiation under varying NPK levels: chlorophyll fluorescence, gas exchange and antioxidants. Physiol Plant 145:474–484

- Sosik HM, Mitchell GB (1991) Absorption, fluorescence, and quantum yield for growth in nitrogen-limited *Dunaliella tertiolecta*. *Limnol oceanograph* 36(5):910–921
- Stitt M (1991) Rising CO<sub>2</sub> levels and their potential significance for carbon flow in plant cells. *Plant Cell Environ* 14:741–762
- 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
- Surabhi GK, Reddy KJ, Singh SK (2009) Photosynthesis, fluorescence, shoot biomass and seed weight responses of three cowpea (*Vigna unguiculata* (L.) Walp) cultivars with contrasting sensitivity to UV-B radiation. *Environ Exp Bot* 66:160–171
- Teramura AH (1983) Effects of ultraviolet-B radiation on the growth and yield of crop plants. *Physiol Plant* 58:415–427
- Ticconi CA, Abel S (2004) Short on phosphate: plant surveillance and countermeasures. *Trends Plant Sci* 9:548–554
- Vergeer LHT, van der Velde G (1997) Phenolic content of daylight-exposed and shaded floating leaves of water lilies (Nymphaeaceae) in relation to infection by fungi. *Oecologia* 112:481–484
- Virgona JM, Farquhar GD (1996) Genotypic variation in relative growth rate and carbon isotope discrimination in sunflower is related to photosynthetic capacity. *Aust J Plant Physiol* 23:227–236
- Willekens H, Camp WV, Van Montagu M, Inze D, Langebartels CH, Sandermann H (1994) Ozone, sulphur dioxide and ultraviolet-B have similar effects on mRNA accumulation of antioxidant genes in *Nicotiana plumbaginifolia* L. *Plant Physiol* 106:1007–1014
- Yao X, Liu Q (2006) Changes in morphological, photosynthetic and physiological responses of Mono Maple seedlings to enhanced UV-B and to nitrogen addition. *Plant Growth Regul* 50:165–177
- Yuichi T, Akizuki M, Shimizu H, Kondo N, Sugahara K (1989) Effect of UV-B (290–320 nm) irradiation on growth and metabolism of cucumber cotyledons. *Physiol Plant* 76:425–430