BIOLOGIA PLANTARUM 58 (2): 328-334, 2014 DOI: 10.1007/s10535-014-0390-0

# Effect of ultraviolet-B radiation on biomass production, lipid peroxidation, reactive oxygen species, and antioxidants in *Withania somnifera*

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## Abstract

The present study was aimed at understanding the effects of long term supplemental UV-B (3.6 kJ $m^{-2}$ $d^{-1}$) on biomass production, accumulation of reactive oxygen species, lipid peroxidation, and enzymatic antioxidants in leaves and roots of *Withania somnifera* (an indigenous medicinal plant). Under the UV-B treatment, a reduction in biomass and an increased malondialdehyde content (a characteristic of lipid peroxidation) were observed in both the shoots and roots. Amongst ROS, $H_2O_2$ content increased under UV-B in the leaves, whereas it decreased in the roots, and superoxide radical production rate decreased in both the plant parts. The activities of all enzymatic antioxidants tested (ascorbate peroxidase, catalase, glutathione reductase, peroxidase, polyphenol oxidase, and superoxide dismutase) increased under the UV-B treatment, the increase being greater in the roots.

*Additional key words*: ascorbate peroxidase, catalase, glutathione reductase, malondialdehyde, oxidative stress, peroxidase, polyphenol oxidase, superoxide dismutase.

## Introduction

Ultraviolet radiation is an abiotic factor which influences various aspects of plant life (Ballaré *et al.* 2011, McKenzie *et al.* 2011) and its effects are highly variable (Krupa and Kickert 1989, Jordan 1996). Plants respond to UV radiation *via* expression of specific genes involved in plant growth, development, and secondary metabolism. Production of biomass, as one of the key parameters, has been studied extensively under UV-B stress (Kumari *et al*. 2009a,b, Ravindran *et al*. 2010). Another definitive response of plants to UV-B exposure is the generation of reactive oxygen species (ROS) like singlet oxygen ($^1O_2$), superoxide radical ($^{\bullet}O_2^-$), $H_2O_2$, and hydroxyl radical ($^{\bullet}OH$). Their over-accumulations can result in cell death (Mackerness *et al.* 2001). To counteract their damaging effects, plants produce ROS scavengers in the form of enzymatic and non-enzymatic antioxidants (Agrawal *et al*. 2009). The former include various enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT), peroxidase (POX), and polyphenol oxidase (PPO) among others (Mittler *et al*. 2004). Fig. 1 summarises some of the effects of UV-B on plant membranes and consequent plant responses.

*Withania somnifera* (*Solanaceae*) is a medicinal plant used for centuries. Its medicinal properties are primarily attributed to withanolides present in its roots (Sharma *et al.* 2011). The effect of UV-B radiation on ROS production and antioxidants is not known in this species. Thus, the present study was aimed at investigating the effect of supplemental UV-B radiation on biomass production, ROS ($H_2O_2$ and $^{\bullet}O_2^-$), and enzymatic antioxidants (APX, CAT, GR, POX, PPO, and SOD) in the leaves and roots of the *W. somnifera* in order to evaluate the significance of the antioxidant systems in conferring tolerance of this plant to UV-B stress.

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*Submitted* 24 May 2013, *last revision* 5 August 2013, *accepted* 30 September 2013.

*Abbreviations*: APX - ascorbate peroxidase; CAT - catalase; DAT - days after transplantation; GR - glutathione reductase; $^{\bullet}OH$ - hydroxyl radical; $H_2O_2$ - hydrogen peroxide; LPO - lipid peroxidation; MDA - malondialdehyde; $^{\bullet}O_2^-$ - superoxide radical; POX - peroxidase; PPO - polyphenol oxidase; ROS - reactive oxygen species; SOD - superoxide dismutase; TBA - thiobarbituric acid; TCA - trichloroacetic acid; UV-B - ultraviolet B radiation; $UV\text{-}B_{BE}$- biologically effective UV-B.

*Acknowledgement*: The authors are thankful to the Head, Department of Botany, Banaras Hindu University, for providing laboratory facilities, and to the University Grants Commission (UGC), New Delhi, for financial assistance.

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## Materials and methods

**Plants and treatments:** The experimental site was located in the Botanical Garden, Department of Botany, Banaras Hindu University, Varanasi (latitude: 25° 18' N, longitude: 82° 03' E, elevation: 76 m above mean sea level), situated in the Eastern Gangetic plains of India. During the experimental period (end of March to mid-July), the average minimum temperature was 24.4 °C and the average maximum temperature was 37.8 °C. Relative humidity ranged from 41.9 to 61.3 %.

One-month-old plants of *Withania somnifera* (L.) Dun. were obtained from nursery and transplanted in field in experimental plots of 1 × 1 m with 12 plants per plot (3 rows with 4 plants in each row at equal distances). The plots were prepared in triplicate for each type of treatment. Spacing between the ridges was 30 cm and the distance between the ridges and plot border was 15 cm. Spacing between the plants was 20 cm. The plants were watered regularly as per the requirement.

Supplemental UV-B radiation was provided using UV-B lamps (*Q Panel UV-B 313* 40 W fluorescent lamps, *Q panel Inc*., Cleveland, OH, USA). Three lamps (120 cm long) were fitted on steel frames at equal distances directly above the plant canopy. For a UV-B treatment, the lamps were covered with a 0.13 mm cellulose diacetate filter (*Cadillac Plastic Co.*, Baltimore, MD, USA) which transmits radiation down to 280 nm, and for control, by a 0.13 mm polyester filter (*Cadillac Plastic Co.*) which absorbs radiation below 320 nm. The control plants received ambient UV-B dose of 9.6 kJ $m^{-2}$ $d^{-1}$ (Caldwell 1971), whereas the treated plants received 9.6 + 3.6 kJ $m^{-2}$ $d^{-1}$. Thus, biologically effective UV-B (UV-$B_{BE}$) simulated 10 % ozone depletion at Varanasi. The filters used in the experiment were changed every week due to their photo-degradation by UV-B. UV-B was provided to the plants after their establishment in the field for 3 h during noon (11:00 to 14:00). The irradiance was measured using *Ultraviolet Intensity Meter* (*UVP Inc*., San Gabriel, CA, USA) and UV-$B_{BE}$ values were determined using *Spectropower-meter* (*Scientech*, Boulder, USA).

The control and treated plants were carefully dug out in the form of monoliths with roots intact, and thoroughly washed with running water. The plant parts were separated and leaves and roots (fresh tissue) were used for all analyses. The sampling was done at 40, 70, and 100 d after transplantation (DAT). Each parameter was analyzed using five replicates for each treatment.

**Analyses:** For biomass determination, plants were collected, their leaves and roots separated, and dried in an oven at 80 °C till a constant mass was achieved. Lipid peroxidation (LPO) was determined in terms of a malondialdehyde (MDA) content measured according Heath and Packer (1968). Hydrogen peroxide content was determined following the method of Alexieva *et al*. (2001) and calculated using a standard curve. Superoxide radical production rate was measured as per the method of Elstner and Hupel (1976) and calculated from a standard graph prepared using potassium nitrite ($KNO_2$).

To analyze enzymatic antioxidants, 400 mg of fresh tissue was homogenized in 10 $cm^3$ of a sodium phosphate buffer (0.1 M, pH 7.0) containing 0.1 % (m/v) *Triton X-100* and 0.2 g of polyvinylpyrrolidone (PVP) under ice-cold conditions. The homogenate was centrifuged at 10 000 *g* for 20 min. The supernatant was collected and re-centrifuged at 13 500 *g* and 4 °C for another 15 min. The supernatant was collected and stored at 4 °C until analysed.

APX was assayed as per the method of Nakano and Asada (1981) after supplementing the extraction buffer with 1 mM ascorbate. A coefficient of absorbance was 2.8 $mM^{-1}$ $cm^{-1}$. CAT activity was determined following the method of Abei (1984) using a coefficient of absorbance of 0.036 $mM^{-1}$ $cm^{-1}$. The method of Anderson (1996) was followed for the determination of a GR activity using the coefficient of absorbance of 6.22 $mM^{-1}$ $cm^{-1}$. POX activity was determined using the method described by Britton and Mehley (1955) and a coefficient of absorbance was 2.47 $mM^{-1}$ $cm^{-1}$. PPO activity was measured according to the method of Kumar and Khan (1982) and one unit was 0.1 change in absorbance per min. SOD activity was determined according to Fridovich (1974) by measuring its ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT), and one unit of the enzyme activity was defined as the amount of the enzyme required for 50 % inhibition of the reduction of NBT.

**Statistics:** The Student's *t*-test was applied to compare the means between the control and treatment for both the leaves and the roots. Two-way *ANOVA* was performed to test the individual and interactive effects of treatments, organs, and age. All statistical analyses was performed using the *SPSS* software *v. 16*.

## Results

A reduction in the leaf as well as root biomass was observed at all the sampling ages in the UV-B treated plants and it was more evident in the roots (Fig. 2). The age and treatment as well as their interaction significantly affected the biomass in both the plant organs (Table 2).

The MDA content (the measure of degree of LPO) increased in the leaves under the UV-B radiation as compared to the controls at all the sampling dates (261.2, 120.2, and 329.8 % at 40, 70, and 100 DAT, respectively; Table 1). In the roots, a similar trend of increment was observed but the MDA content was lower in the UV-B treated roots as compared to the leaves. Results of two-

way *ANOVA* indicate that the degree of LPO was significantly affected by the treatment, age, and their interaction (Table 2).

The UV-B treated leaves recorded an increase in the $H_2O_2$ content compared to the control ones at all the sampling dates, whereas a reduction was observed in the roots (Table 1). The $^{\bullet}O_2^-$ production rate decreased in the UV-B treated leaves compared to the control ones (Table 1) and a maximum reduction was observed at 40 DAT (46.3 %, $P$ < 0.001). In the roots, a similar trend of decrease was observed (43.4, 21.7, and 23.6 % at 40, 70, and 100 DAT, respectively). When comparing the leaves and roots of the control and UV-B treated plants, the $^{\bullet}O_2^-$ production rate was higher in the leaves than in the roots (Table 1). The $^{\bullet}O_2^-$ production was significantly affected by all individual factors as well as their interactions (Table 2).

Under the UV-B treatment, the APX activity was higher in the leaves at 40 and 70 DAT and the in roots at all three ages compared to the control, however, it was lower in the leaves at 100 DAT. The treated plants showed a higher APX activity in the roots compared to the leaves at all three sampling dates (Table 1). The APX activity varied significantly with the plant age, treatment, and their interaction (Table 2).

The leaf and root CAT activities were higher under the UV-B treatment than in the control at all three sampling dates, though an increase in the leaves was insignificant at 40 DAT (11.63 %). The CAT activity was higher in the leaves compared to the roots in the control plants, but in the UV-B treated plants, roots possessed a higher CAT activity compared to the leaves at 40 and 70 DAT. At 100 DAT, the CAT activity was higher in the UV-B exposed leaves as compared to the roots

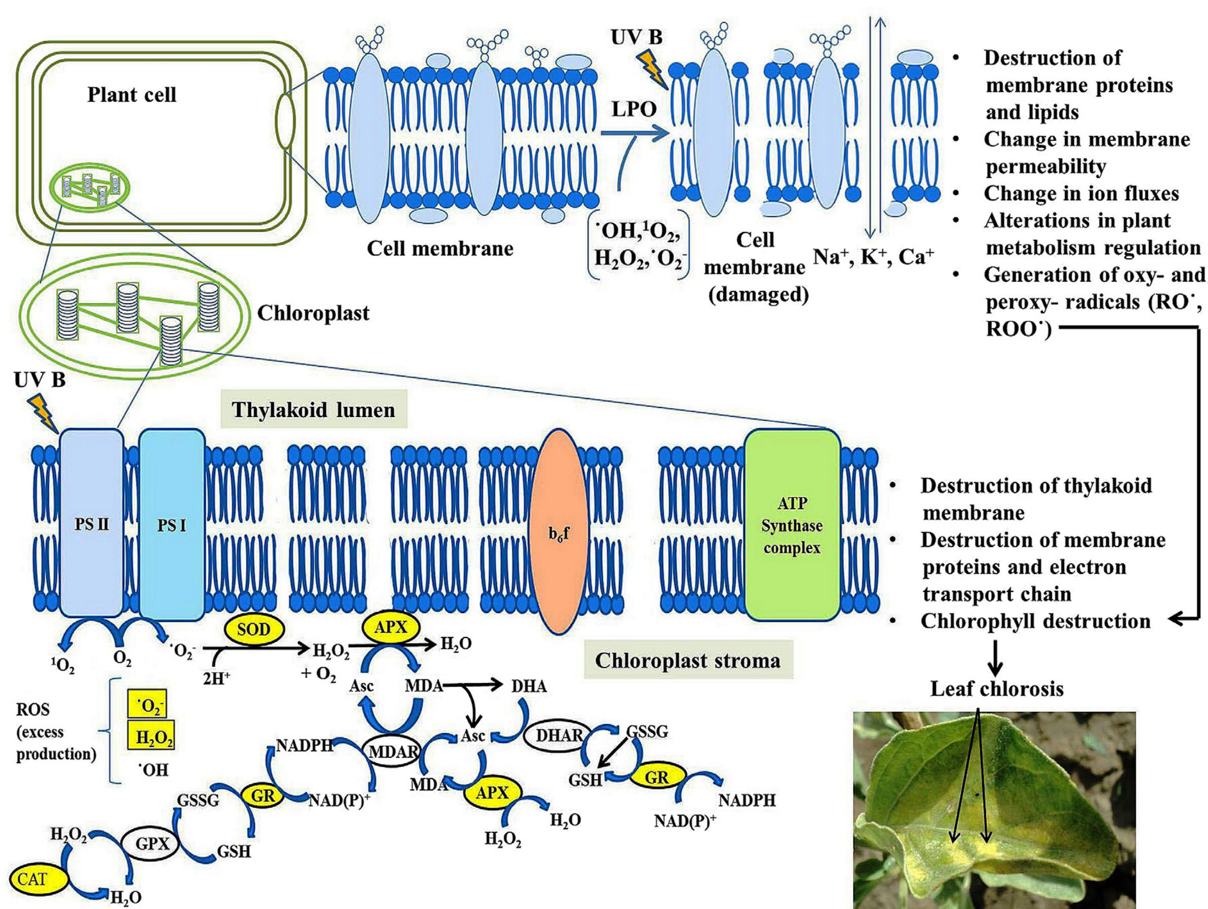


Fig. 1. Under UV-B radiation stress, different ROS are formed by photosystems in the thylakoid membrane of chloroplast and leads to the activation of complex antioxidant defense system which includes the enzymes of the Haliwell-Asada pathway (Mittler *et al.* 2004). At PS II, $^{\bullet}O_2^-$, $H_2O_2$, $^1O_2$, and $^{\bullet}OH$ are produced from molecular oxygen. $^1O_2$ can also be produced at PS I. $^{\bullet}O_2^-$ is dismutated by SOD to $H_2O_2$. $^1O_2$ is highly reactive and has a short half-life. It causes degradation of D1 protein and loss of PS II activity (photoinhibition). Unquenched $^1O_2$ also causes lipid peroxidation. $^1O_2$ and $^{\bullet}OH$ can be scavenged by Asc, Toc, and GSH. $H_2O_2$ is scavenged by Asc, GSH, and POXs. Not all chloroplast and membrane damage reactions are shown here. Abbreviations: APX - ascorbate peroxidase; Asc - ascorbate; CAT - catalase; DHA - dehydroascorbate; DHAR - dehydroascorbate reductase; GR - glutathione reductase; GSH - reduced glutathione; GSSG - oxidized glutathione; LPO - lipid peroxidation; MDA -malondialdehyde; MDAR - malondialdehyde reductase; $^1O_2$ - singlet oxygen; $^{\bullet}OH$ - hydroxyl radical; POXs - peroxidases; PS I - photosystem I; PS II - photosystem II; SOD - superoxide dismutase; Toc - tocopherol.

(Table 1). The CAT activity varied significantly with the plant age, treatment, and their interactions (Table 2).

The GR activity in the UV-B treated plants increased initially by 110.5 and 155.8 % at 40 and 70 DAT, respectively, compared to the control ones, but it declined as the leaves matured (11.7 %), though this reduction was non-significant (Table 1). In the roots, the GR activity was higher compared to the control. In the control plants, the GR activity was higher in the roots at 40 and 70 DAT, whereas in the UV-B treated plants, the GR activity was higher in the roots as compared to the leaves at all the sampling dates (Table 1). The plant age as well as the treatment affected the GR activity significantly (Table 2).

The POX activity of the UV-B treated plants of all three ages showed an enhancement in the leaves and roots. A higher POX activity was always in the roots than in the leaves (Table 1). The plant age, UV-B treatment and their interactions significantly affected the POX activity (Table 2).

The UV-B treated leaves showed enhanced PPO when compared with the control. A maximum increment was observed at 100 DAT (431.0 %, $P < 0.001$). A similar trend was observed in the roots with the maximum increase of the PPO activity being at 70 DAT (70.4 %). In

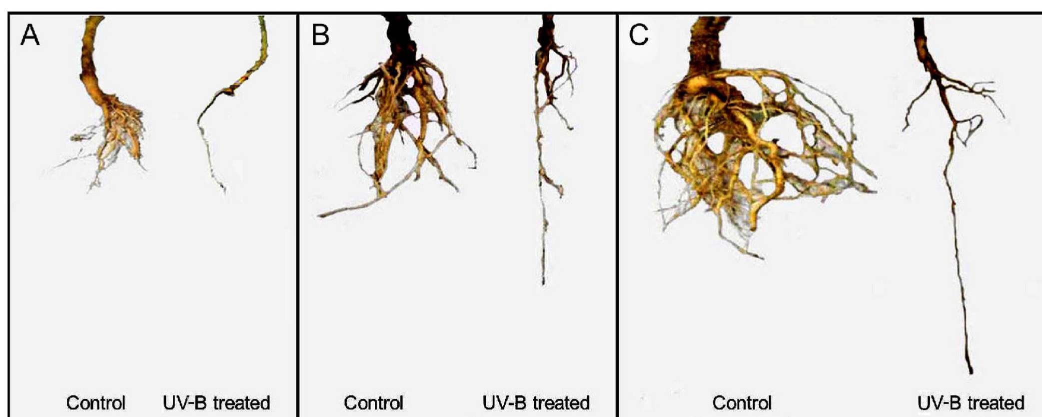


Fig. 2. Control and UV-B treated roots of *W. somnifera* at 40 (*A*), 70 (*B*), and 100 (*C*) days after transplantation.

Table 1. The effects of UV-B treatment on the biomass [g plant$^{-1}$], content of MDA [nmol g$^{-1}$(f.m.)] and $H_2O_2$ [μmol g$^{-1}$(f.m.)], $^{\bullet}O_2^-$ production rate [nmol g$^{-1}$(f.m.) min$^{-1}$], and activities of APX, CAT, GR [mmol g$^{-1}$(f.m.) min$^{-1}$], POX [μmol g$^{-1}$(f.m.) min$^{-1}$], PPO, and SOD [U g$^{-1}$(f.m.)] in leaves and roots of *Withania somnifera* at three sampling dates. Means ± SE $n$ = 5. Differences significant at * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$. DAT - days after transplantation.

| Organs | Parameter | 40 DAT control | UV-B | 70 DAT control | UV-B | 100 DAT control | UV-B |
|---|---|---|---|---|---|---|---|
| Leaves | biomass | 1.67 ±0.008 | 0.64 ±0.012$^{**}$ | 2.03 ±0.067 | 1.23 ±0.033$^{**}$ | 4.50 ±0.058 | 2.33 ±0.033$^{**}$ |
| | MDA | 0.32 ±0.007 | 1.14 ±0.152$^{*}$ | 1.00 ±0.006 | 2.21 ±0.019$^{**}$ | 1.05 ±0.014 | 4.50 ±0.092$^{***}$ |
| | $H_2O_2$ | 0.49 ±0.006 | 0.58 ±0.008$^{***}$ | 0.61 ±0.002 | 0.97 ±0.021$^{***}$ | 0.94 ±0.006 | 1.03 ±0.021$^{**}$ |
| | $^{\bullet}O_2^-$ | 0.13 ±0.004 | 0.07 ±0.003$^{***}$ | 0.18 ±0.001 | 0.16 ±0.001$^{***}$ | 0.27 ±0.002 | 0.20 ±0.001$^{***}$ |
| | APX | 0.016±0.003 | 0.019±0.004$^{**}$ | 0.024±0.002 | 0.028±0.001$^{***}$ | 0.058±0.060 | 0.037±0.024$^{***}$ |
| | CAT | 1.51 ±0.029 | 1.69 ±0.036$^{ns}$ | 1.87 ±0.040 | 5.41 ±0.029$^{***}$ | 3.72 ±0.022 | 4.38 ±0.029$^{***}$ |
| | GR | 0.002±0.000 | 0.005±0.000$^{*}$ | 0.003±0.000 | 0.006±0.000$^{**}$ | 0.014±0.000 | 0.013±0.000$^{ns}$ |
| | POX | 84.56 ±0.165 | 85.63 ±0.218$^{ns}$ | 101.76 ±0.428 | 117.41 ±0.297$^{**}$ | 154.54 ±0.460 | 193.54 ±0.110$^{***}$ |
| | PPO | 0.016±0.001 | 0.037±0.006$^{*}$ | 0.024±0.002 | 0.108±0.000$^{***}$ | 0.026±0.000 | 0.140±0.002$^{***}$ |
| | SOD | 1.06 ±0.006 | 1.172±0.014$^{ns}$ | 2.03 ±0.006 | 2.99 ±0.008$^{***}$ | 2.537±0.004 | 3.56 ±0.007$^{***}$ |
| Roots | biomass | 0.23 ±0.067 | 0.15 ±0.029$^{*}$ | 1.33 ±0.006 | 0.63 ±0.009$^{**}$ | 4.22 ±0.002 | 1.81 ±0.003$^{***}$ |
| | MDA | 0.57 ±0.013 | 0.84 ±0.004$^{**}$ | 0.91 ±0.013 | 1.62 ±0.013$^{**}$ | 1.25 ±0.028 | 3.56 ±0.018$^{***}$ |
| | $H_2O_2$ | 0.48 ±0.006 | 0.35 ±0.014$^{***}$ | 0.85 ±0.054 | 0.51 ±0.006$^{***}$ | 0.90 ±0.026 | 0.63 ±0.017$^{**}$ |
| | $^{\bullet}O_2^-$ | 0.09 ±0.002 | 0.05 ±0.004$^{***}$ | 0.15 ±0.003 | 0.12 ±0.002$^{***}$ | 0.21 ±0.002 | 0.16 ±0.001$^{***}$ |
| | APX | 0.02 ±0.001 | 0.03 ±0.000$^{**}$ | 0.03 ±0.001 | 0.04 ±0.000$^{***}$ | 0.06 ±0.001 | 0.06 ±0.000$^{**}$ |
| | CAT | 1.26 ±0.080 | 6.74 ±0.048$^{***}$ | 1.76 ±0.011 | 10.53 ±0.107$^{***}$ | 2.55 ±0.030 | 3.12 ±0.017$^{**}$ |
| | GR | 0.006±0.000 | 0.015±0.000$^{**}$ | 0.008±0.000 | 0.018±0.000$^{***}$ | 0.009±0.000 | 0.026±0.001$^{**}$ |
| | POX | 91.31 ±0.218 | 94.11 ±0.285$^{ns}$ | 122.67 ±0.218 | 136.76 ±0.359$^{***}$ | 183.98 ±0.167 | 204.27 ±0.303$^{***}$ |
| | PPO | 0.021±0.001 | 0.043±0.001$^{**}$ | 0.035±0.002 | 0.120±0.002$^{**}$ | 0.067±0.000 | 0.203±0.001$^{***}$ |
| | SOD | 3.42 ±0.008 | 4.19 ±0.009$^{***}$ | 3.8 5±0.011 | 4.66 ±0.012$^{***}$ | 4.15 ±0.013 | 5.81 ±0.023$^{***}$ |

both the control and treated plants, activity was higher in the roots than in the leaves (Table 1). Two-way *ANOVA* showed significant variations in the PPO activity with respect to the age, treatment and their interaction (Table 2).

The UV-B treatment enhanced the SOD activity in the leaves and roots at all three sampling dates. The SOD activity increased with the age in control as well as in the UV-B treated leaves and roots (Table 1). Results of two-way *ANOVA* showed that there was a significant variation in the SOD activity at different plant ages, treatments, and their interactions (Table 2).

## Discussion

A reduction in biomass under a UV-B stress has been reported in a majority of studies although in some instances it remains unchanged or increases (Zhao *et al.* 2013).

Table 2. Two-way *ANOVA* test to determine the effects of the UV-B (T), plant age (A), and their interactions on the biomass, lipid peroxidation (MDA), ROS, and antioxidant enzymes in *W. somnifera* leaves and roots. *F* ratios and levels of significance (*** - $P < 0.001$).

| Organ | Parameter | A | T | A×T |
|---|---|---|---|---|
| Leaves | biomass | 952.8*** | 890.3*** | 89.8*** |
| | MDA | 391.6*** | 932.2*** | 187.6*** |
| | $H_2O_2$ | 134.1*** | 66.5*** | 15.9*** |
| | $^{\bullet}O_2^-$ | 1863 *** | 917.9*** | 86.9*** |
| | APX | 46720 *** | 2562 *** | 10080 *** |
| | CAT | 3421 *** | 3164 *** | 1646 *** |
| | GR | 1211 *** | 70.9*** | 81.8*** |
| | POX | 56180 *** | 6740 *** | 2366 *** |
| | PPO | 242.6*** | 1157 *** | 162.9*** |
| | SOD | 75810 *** | 27720 *** | 4881 *** |
| Roots | biomass | 4081 *** | 1623 *** | 699.0*** |
| | MDA | 5572 *** | 6681 *** | 2145 *** |
| | $H_2O_2$ | 2909 *** | 4418 *** | 517.0*** |
| | $^{\bullet}O_2^-$ | 2550 *** | 837.2*** | 20.4*** |
| | APX | 35620 *** | 8351 *** | 181.2*** |
| | CAT | 1567 *** | 10200 *** | 2367 *** |
| | GR | 188.1*** | 1459 *** | 73.3*** |
| | POX | 56110 *** | 2552 *** | 455.4*** |
| | PPO | 3391 *** | 6176 *** | 1047 *** |
| | SOD | 5712 *** | 14490 *** | 1053 *** |

A greater decrease in the root biomass compared to the leaf biomass might be because the roots utilized a great amount of photosynthates for the production of anti-oxidative defense enzymes preventing root oxidative damage. Photosynthates might also be channelized towards the production of higher quantities of important secondary metabolites. The plant response to UV-B is likely dependent on shoot to root signaling which may involve phytohormones, ROS, calcium, cyclic nucleotides, nitric oxide, sugars, abscisic acid, jasmonates, salicylic acid, and polyamines (Stratmann 2003, Ktitorova *et al*. 2006, Krasylenko *et al*. 2012). Cross-talk between these signaling pathways is a common plant stress response affecting both its growth and metabolism. A higher decrease in the root biomass was also observed previously in wheat exposed to UV-B (Agrawal *et al.* 2004). *W. somnifera* is a medicinal plant producing a number of pharmaceutically important secondary metabolites. Under a UV-B stress, a decrease in the plant biomass is usually accompanied by an increase in secondary metabolite production (Zhang and Björn 2009). Hence, the economic yield loss might be less than anticipated based only on biomass reduction.

The lipids in cell membranes were destroyed due to excessive ROS generation during the UV-B stress depicted by an increase in the MDA content in the treated plants of *W. somnifera*. Lipid peroxidation is a self-propagating chain reaction, hence initial oxidation of only a few lipid molecules often results in substantial tissue damage. Alkoxy- and peroxy-radicals, products of lipid peroxidation, are known to destroy chlorophyll (Peiser and Yang 1978) (Fig. 1). The results obtained in this study are corroborated by several other findings made by Kumari *et al.* (2010) in *Acorus calamus*, Tripathi *et al.* (2011) in *Linum usitatissimum*, and Hagh et al. (2012) in sunflower cultivars**.**

An increase in the $H_2O_2$ content found in the leaves of UV-B treated *W. somnifera* plants has been reported also in other plants (Rybus Zając 2005, Kubiś and Rybus Zając 2008). An increase in the SOD activity is one of the reasons for higher production of $H_2O_2$ in plants. $H_2O_2$ is scavenged by catalase, various peroxidases, and enzymes of ascorbate-glutathione cycle. The higher activities of CAT, APX, and GR in the roots might be responsible for more efficient quenching of $H_2O_2$ in the roots of the treated plants. $H_2O_2$ reacts with $^{\bullet}O_2^-$ *via* Fenton reaction to produce $^{\bullet}OH$ which is the most reactive ROS and may be responsible for higher lipid peroxidation in the UV-B exposed leaves compared to the roots.

In our study, the rather low $^{\bullet}O_2^-$ production rate might be primarily due to the high SOD activity. An activation of SOD in plants under a UV-B treatment has been reported by Rao and Ormrod (1995). SOD remove $^{\bullet}O_2^-$ by catalyzing its dismutation to $H_2O_2$ and $O_2$ (Agarwal 2007, Gill and Tuteja 2010). Also, SOD sprayed *Arabidopsis* leaves showed lower production of $^{\bullet}O_2^-$ than control ones under a UV-B treatment (Mackerness *et al*. 2001).

APX plays an important role in scavenging $H_2O_2$ produced by SOD. It is required to maintain the redox state of cells under stress (Asada 1992). Its increased activity was observed both in the leaves and roots of the treated plants as compared to the control ones. An increased APX activity has been also observed in cucumber (Kataria *et al.* 2007), kidney bean (Singh *et al.* 2011), and sunflower (Hagh *et al.* 2012). However, a

decline in the APX activity in the treated plants at 100 DAT might probably be due to APX degradation or repression of *APX* gene expression under prolonged UV-B exposure (Casati *et al.* 2002).

CAT directly dismutates $H_2O_2$ into $H_2O$ and $O_2$ and is an important ROS scavenging enzyme during stress conditions (Garg and Manchanda 2009, Gill and Tuteja 2010). In agreement with our experiments, CAT activity was reported to increase under UV-B by Balakumar *et al.* (1997), and Hagh *et al.* (2012). Results similar to ours were also observed by Singh (1996) in UV-B treated *Glycine max*, and Kumari *et al.* (2010) in *Acorus calamus*, a medicinal plant. However, this enzyme is susceptible to photoinactivation and degradation, hence its activity is reduced under prolonged and high irradiance. Also, it has poor affinity for $H_2O_2$ and hence it is limited in effectiveness (Shim *et al*. 2003). A decreased CAT activity could also be due to the destruction of peroxisomes due to high lipid peroxidation under a UV-B stress (Ravindran *et al*. 2010). POX decomposes $H_2O_2$ by oxidation of co-substrates (Gaspar *et al*. 1991). UV-B radiation increased POX activity in several plant species including wheat and mung bean (Agrawal and Rathore 2007), peanut (Tang *et al.* 2010), and cucumber (Hagh *et al*. 2012). Nandi *et al.* (1984) suggested that the inverse relationship between CAT and POX_might be due to disintegration of tetrameric catalase molecules under stress into monomeric units having peroxidase-like activity.

GR is an important enzyme of the ascorbate-glutathione cycle (Rao and Reddy 2008). An increase in GR activity has been found in the earlier reports of Xu *et al.* (2008) and Cakirlar *et al.* (2011). The reduced APX activity and the unchanged GR activity at 100 DAT in the leaves indicate reduced efficiency of the ASH-GSH cycle to combat oxidative stress. An increase in PPO activity under UV-B radiation has been observed in many studies (Ravindran *et al.* 2008, Indrajith and Ravindran 2009). PPO is responsible for the oxidation of phenolic compounds to quinones (Thypyapong *et al*. 1995) and may play an important role in preventing oxidative damage due to UV-B radiation.

In conclusion, UV-B radiation induced antioxidative defenses protecting *W. somnifera* plants against deleterious effects of ROS. UV-B affected both the leaves and roots in a similar manner for almost all the parameters tested and at all the sampling dates.

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