Indian Journal of Experimental Biology
Vol. 55, December 2017, pp. 845-852

Effect of supplemental ultraviolet-B radiation on *Withania somnifera* L. (Dunal): An appraisal of morphological, physiological and biochemical characteristics

S Takshak & SB Agrawal*

Laboratory of Air Pollution and Global Climate Change, Department of Botany,
Banaras Hindu University, Varanasi-221 005, Uttar Pradesh, India

Received 03 August 2015; revised 26 August 2016

Withania somnifera L. (Dunal), commonly called Indian Ginseng or Ashwagandha, is an indigenous medicinal plant and has been a source of numerous pharmacological compounds for centuries, and ultraviolet-B (UV-B) radiation is one of the potent factors capable of influencing the plant's performance, consequently influencing its medicinal properties. UV-B studies on medicinal plants are scarce, especially with respect to the overall plant performance. In the present study, we subjected *W. somnifera* to supplemental UV-B (s-UV-B) radiation (+3.6 kJ m^{-2} day^{-1}) above ambient to study its morphological, physiological, and biochemical characteristics at three sampling ages (40, 70 and 100 days after transplantation). All growth characteristics, except root length, reduced under s-UV-B treatment. Physiological parameters, such as photosynthetic rate (P_s), stomatal conductance (g_s) and water use efficiency (WUE) decreased under s-UV-B while internal CO_2 (C_i) increased. Quantum yield (F_v/F_m) also recorded a decline at all sampling ages. Protein and chlorophyll contents decreased while phenolics and antioxidants (ascorbic acid, α-tocopherol and thiols) increased in exposed leaves and roots. IAA oxidase activity also increased in both plant parts at all ages. The study concludes that s-UV-B stress is a potent factor in increasing the concentrations of defence compounds and antioxidants to optimise the plant performance under this stress.

Keywords: Antioxidants, Ascorbic acid, Ashwagandha, Chlorophyll, IAA oxidase activity, Indian ginseng, Phenolics, Photosynthesis, Stratospheric ozone, Stress, α-Tocopherol, Thiols, UV-B, Vitamin C, Vitamin E

Depletion of stratospheric ozone (O_3) layer is considered to be the most direct cause of biologically active ultraviolet-B (UV-B) reaching the Earth's surface in enhanced quantities and posing deleterious effects on natural plant ecosystems[1]. Though effective controls imposed upon the release of O_3 depleting substances are in place, recent studies[2,3] have indicated that increased levels of UV-B reaching the Earth might not be so easily mitigated. Plants, being sessile, are inevitably exposed to UV-B radiation and hence, adapt themselves as per the altered conditions. These adaptations manifest themselves in terms of changes in plant architecture (altered morphological traits), physiological characteristics, biochemical and genetic level changes[4,5]. On the biochemical front, alterations in both primary (e.g. proteins) and secondary (e.g. phenolics) metabolites have been observed in plants[4]. Excess generation of reactive oxygen species (ROS) are countered via enzymatic and nonenzymatic antioxidants. The latter category primarily includes ascorbic acid and α-tocopherol[6] amongst others. Other small molecules such as thiol and proline may also serve as antioxidants in plants under stress. These metabolites are also implicated in vital plant functions, such as photosynthate assimilation and transport, gene expression, regulation of transcript accumulation, enzyme activity regulation, and influencing protein structure and stability[7]. The levels of free indole-3-acetic acid (IAA) in plants are regulated via their degradation by IAA oxidase (IAAO); IAAO activity has been shown to be inversely correlated with IAA concentrations[8]. Thus, IAAO, via regulating IAA levels, are implicated in optimizing plant growth and development under stress conditions.

Withania somnifera (Solanaceae), commonly called Indian ginseng or Ashwagandha, is an important medicinal plant for the withanolides present in its roots which is used in traditional Indian medicine for

*Correspondence:
Phone: +91 542 2368156; Fax: +91 542 2368174
E-mail: sbagrawal56@gmail.com

Abbreviations: AA, ascorbic acid; C_i, internal CO_2; DAT, days after transplantation; F_0, initial fluorescence; F_m, maximum fluorescence; F_v, variable fluorescence; g_s, Stomatal conductance; IAA, Indole-3-acetic acid; IAAO, IAA oxidase; O_3, ozone; P_s, Photosynthetic rate; ROS, Reactive oxygen species; UV-B, Ultraviolet-B; WUE, Water use efficiency

centuries[9,10]. A majority of studies on medicinal plants focus on the isolation of the pharmacologically important constituents[10] and studying their health benefits; studies on the morphological, physiological, and biochemical aspects of medicinal plants (in order to determine the overall plant performance) are comparatively scarce. Hence, in the present study we evaluated the morphological, physiological, and biochemical aspects of this indigenous medicinal plant to determine its performance under supplemental UV-B radiation stress.

Materials and Methods

Experimental site

The experiment was carried out under near-natural field conditions in the Botanical Garden, Department of Botany, Banaras Hindu University, Varanasi (25° 18 N, 82° 03' E, and 76 m above mean sea level), India. The experimental period extended from end of March to mid-July. The average maximum and minimum temperatures recorded were 37.8 and 24.4°C, respectively while relative humidity ranged from 41.9 to 61.3%.

Experimental design

Withania somnifera plants (one month old, obtained from nursery) were transplanted in field experimental plots of 1×1 m with 3 rows in each plot and 4 plants per row (hence 12 plants per plot) at equal distances. The plots were prepared in triplicate for each type of treatment. The spacing between the ridges, between ridges and plot border, and between plants was kept at 30, 15, and 20 cm, respectively. The plants were watered regularly as per the requirements.

s-UV-B treatment

After the plants were established in the field, they were exposed to supplemental UV (s-UV-B) radiation using UV-B lamps (Q Panel UV-B 313 40 W fluorescent lamps, Q panel Inc., Cleveland, OH, USA). The lamps were fitted on steel frames directly above the plant canopy at a distance of 30 cm; the distance between the lamps and the plant canopy was kept constant throughout the experiment. 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 for s-UV-B treatment while for control, 0.13 mm polyester filter (Cadillac Plastic Co.) which absorbs radiation below 320 nm was used. Thus, control plants received only ambient UV-B dose (9.6 kJ m^{-2} d^{-1}), while treated plants received ambient+3.6 kJ m^{-2} d^{-1} UV-B (biologically effective UV-B (UV-B_{BE}) simulating 10% ozone depletion over Varanasi) as weighted by Caldwell generalized plant action spectrum normalized at 300 nm[11]. The filters were replaced every week due to their photodegradation by UV-B. UV-B treatment was given to the plants during the solar noon period (11:00 a.m. to 2:00 p.m.). UV-B irradiance was measured using Ultraviolet Intensity Meter (UVP Inc., San Gabriel, CA, USA), while UV-B_{BE} values were determined using Spectropower-meter (Scientech, Boulder, USA).

Plant sampling and analysis

For various analyses, the plants were dug out in the form of monoliths, with roots intact and thoroughly washed with running water, and plant parts separated. Fresh tissue (leaves and roots) were used for all analyses. Sampling was done at 3 ages [40, 70, and 100 DAT (days after transplantation)].

Measurement of growth traits

For biomass determination, the plants were collected, the plant parts separated, and oven dried at 80°C till constant weight was achieved. Dry weights of different plant parts were added to obtain the total biomass of each plant. It was expressed in terms of g $plant^{-1}$. Other growth characteristics (root length, shoot length, plant height, number of leaves, leaf area, root girth, number of flowers and number of fruits) were also determined. The total leaf surface area was measured using a portable leaf area meter (Model Li-3100, Li-COR, Inc., USA).

Determination of physiological parameters

Photosynthetic rate (P_s), stomatal conductance (g_s), and internal CO_2 (C_i) were measured using a portable photosynthetic system (Model LI6400 XT, Version 6.2, Lincoln, Nebraska, USA) at the three sampling ages (40, 70, and 100 DAT). Water use efficiency (WUE) was calculated as the ratio of photosynthetic rate to transpiration. The measurements were made between 9:00 and 10:30 a.m. on the third fully expanded leaf from top of randomly selected plants of each treatment.

Chlorophyll fluorescence characteristics, initial fluorescence (F_0) and maximum fluorescence (F_m) were measured from which variable fluorescence (F_v) and photochemical efficiency (F_v/F_m) were calculated. Measurements were made using plant efficiency analyzer (Model PEA, MK2-9414, Hansatech Instruments Ltd., UK) on the same leaves on which photosynthesis was measured during the same hours. The leaves were subjected to dark adaptation for 30

min on the adaxial side using leaf clips. The leaf surface was then irradiated with red light and fluorescence signal collected at excitation irradiance set at 3000 μmol m^{-2} s^{-1}.

Determination of plant metabolites

Protein, phenol, thiol, proline, ascorbic acid, α-tocopherol, and chlorophyll contents along with IAAO activity were determined using the standard methods previously reported in detail[12].

Statistical analysis

Growth characteristics

A significant reduction in total biomass was observed at three sampling ages (Table 1). The age and treatment as well as their interactions significantly affected plant biomass (Table 2). Root length had increased significantly at all three ages, maximum increment being recorded at 40 DAT (45.2%, P <0.01) while shoot length decreased under s-UV-B treatment (28.0, 24.7, and 25.7% at 40, 70 and 100 DAT, respectively) (Table 1). The ANOVAR results indicate that the root length was significantly affected by age and treatment while shoot length was significantly affected by both the individual factors and their interaction (Table 2). Total plant height decreased at all ages though the decrease was not significant at first two sampling ages (6.25 and 10.2%). Plant age, s-UV-B treatment and their interactions affected this parameter significantly (Table 2).

With the increase in root length, root girth decreased significantly by 32.8, 34.8 and 40.0% at 40, 70 and 100 DAT, respectively (Table 1). It varied significantly with plant age, treatment and their interactions (Table 2). Number of leaves and leaf area both declined significantly under s-UV-B treatment (Table 1). ANOVAR showed significant variation in the number of leaves and leaf area with respect to age, treatment, and their interactions (Table 2). Number of flowers also declined in treated plants with maximal reduction being observed at 40 DAT (60.7%, P <0.01) and minimal at 100 DAT (40.6%, P <0.01) (Table 1). Number of fruits also recorded a reduction of 44.6 and 48.6% at 70 and 100 DAT, respectively. Both these parameters varied significantly with plant age, treatment, and their interactions, as depicted by the results of ANOVAR (Table 2).

Physiological parameters

P_s, g_s and WUE were adversely affected under s-UV-B treatment at all ages, while C_i increased. Maximum reduction in P_s and WUE was observed at 40 DAT (54.4 and 32.5%, respectively), while g_s declined maximally at 100 DAT (37.3%) (Table 1). Significant increment in C_i was observed at all sampling ages (16.9, 11.0 and 13.3% at 40, 70 and 100 DAT, respectively). Plant age and treatment affected all of these characteristics significantly (except UWE with age) while the effect due to the interactions was less (for g_s and WUE) or nonsignificant (for P_s and C_i) compared to the effect of individual factors (Table 2). s-UV-B lead to an increase in F_0 by 42.7, 39.7 and 38.3% at 40, 70, and 100 DAT, respectively while F_m declined by 20.9, 22.7 and 27.4% at these respective ages (Table 1). Consequently, F_v ($=F_m-F_0$) also declined significantly at all ages. F_v/F_m (a measurement of quantum yield) also reduced under s-UV-B by 17.6, 17.5 and 20.3% at 40, 70 and 100 DAT respectively (Table 1). F_0 was significantly affected by individual factors of age and treatment but not their interactive effects while F_v/F_m was significantly affected only due to s-UV-B treatment as depicted by the results of ANOVAR (Table 2).

Metabolites and IAA oxidase

Under s-UV-B treatment, both the leaves and roots of the test plant showed a decrease in protein content at all three sampling ages with maximal decrease being recorded at 40 DAT in roots (58.0%, P <0.001). In both control and treated plants, the protein content was higher in roots compared to that of leaves (Table 1). As per the results of ANOVAR, plant age, UV-B treatment, and their interactions significantly affected protein content (Table 2). Phenol content in both plant organs showed an enhancement under UV-B treatment as compared to their respective controls. Leaves and roots showed maximum increment at 40 DAT (18.8%, P <0.001) and 100 DAT (32.3%, P <0.001), respectively. Roots recorded a higher amount of phenolics under control as well as treatment conditions (Table 1). Phenol content was significantly affected by all individual factors as well as their interactions (Table 2).

Compared to the control leaves, s-UV-B treated leaves showed increased thiol content at all sampling ages (32.4, 17.7 and 8.8% at 40, 70 and 100 DAT respectively, P <0.001). A similar trend was also observed in roots with maximum increase being recorded at 40 DAT (68.0%, P <0.001). Higher amount of thiol was observed in leaves compared to the roots in both control and treated plant organs (Table 1). Age, treatment, and their interactions significantly affected thiol content in leaves while the interactive effect was

Table 1 — The effect of s-UV-B treatment on (A) growth and physiological characteristics in; and (B) metabolites in leaves and roots of *Withania somnifera* at three sampling ages. [Means±SE, n=5 (growth/leaves/roots); n=15 (physiology). Differences significant at * P <0.05, ** P <0.01, *** P <0.001, ns= non-significant. DAT-days after transplantation]

Growth Parameters	40 DAT		70 DAT		100 DAT	
	Control	s-UV-B	Control	s-UV-B	Control	s-UV-B
Total biomass (g plant^{-1})	4.29±0.189	3.05±0.048**	9.10±0.221	6.53±0.081***	22.01±0.531	15.49±0.356***
Root length (cm)	9.02±0.143	13.10±0.733**	10.34±0.375	14.54±0.349***	18.58±0.146	22.10±0.406**
Shoot length (cm)	21.36±0.798	15.38±0.360**	36.34±1.380	27.38±0.609**	54.14±1.385	40.24±0.720***
Plant height (cm)	30.38±0.887	28.48±0.980ns	46.68±1.728	41.92±0.881ns	72.72±1.302	62.34±0.926***
Number of leaves	58.20±2.396	35.00±1.304**	106.20±3.992	60.80±2.577**	373.20±7.768	238.40±6.698**
Leaf area (cm^{-2})	59.85±2.619	46.64±2.087*	125.74±3.017	102.34±4.244*	362.18±5.388	327.22±9.387**
Root girth	2.38±0.086	1.60±0.105**	3.68±0.086	2.40±0.144**	5.70±0.105	3.42±0.107***
Number of flowers	12.20±0.917	4.80±0.374**	29.80±1.594	11.60±0.812***	85.60±3.723	46.20±1.655**
Number of fruits	-	-	11.20±1.393	6.20±1.356**	166.60±4.155	85.60±2.227***
Physiological parameters						
P_s (μmol CO_2 m^{-2} s^{-1})	14.84±0.325	6.78±0.301***	17.62±0.251	11.28±0.451***	23.57±0.364	15.87±0.297***
g_s(mol H_2O m^{-2} s^{-1})	1.19±0.048	0.80±0.009***	2.15±0.048	1.82±0.016***	2.51±0.120	1.58±0.075***
C_i(μmol mol^{-1})	286.19±3.149	334.44±2.397***	337.58±1.850	374.85±1.883***	315.74±0.887	357.73±1.513***
WUE(μmol CO_2 m^{-2} s^{-1}/ mmol m^{-2} s^{-1})	5.25±0.174	3.54±0.186***	3.10±0.100	2.62±0.111**	4.65±0.206	3.95±0.134**
F_0	215.13±1.341	307.00±12.493***	222.80±2.024	311.33±1.450***	253.93±2.456	351.27±2.546***
F_m	1193.27±30.940	943.53±8.487***	1254.40±16.386	969.20±12.426***	1393.07±14.421	1011.47±16.719***
F_v	978.13±31.372	636.53±16.933***	1031.60±15.111	657.87±11.897***	1139.13±15.026	660.20±16.308***
F_v/F_m	0.818±0.005	0.647±0.015***	0.822±0.002	0.678±0.004***	0.817±0.003	0.652±0.006***
Leaves						
Protein (mg g^{-1} f.w.)	1.573±0.005	0.771±0.007***	4.758±0.011	2.212±0.042***	5.786±0.002	4.406±0.009***
Phenol (mg g^{-1} f.w.)	3.574±0.022	4.244±0.043***	5.196±0.031	6.066±0.037***	9.772±0.049	10.596±0.013***
Thiol (mg g^{-1} f.w.)	5.390±0.065	7.136±0.018***	6.385±0.045	7.512±0.031***	6.765±0.022	7.358±0.018***
Proline (mg g^{-1} f.w.)	0.510±0.014	0.849±0.013***	0.052±0.041	0.961±0.010**	0.844±0.014	1.049±0.012***
Ascorbic acid (mg g^{-1} f.w.)	0.069±0.004	0.132±0.002***	0.119±0.001	0.189±0.003***	0.143±0.001	0.266±0.001***
α-tocopherol (mg g^{-1} f.w.)	5.21±0.034	6.17±0.017***	8.82±0.017	9.62±0.009***	10.63±0.022	11.45±0.025***
Chlorophyll a (mg g^{-1} f.w.)	0.783±0.006	0.633±0005***	1.231±0.002	0.860±0.003***	1.509±0.002	0.578±0.002***
Chlorophyll b (mg g^{-1} f.w.)	0.221±0.006	0.122±0.002***	0.336±0.003	0.164±0.004***	0.082±0.002	0.216±0.003***
Total chlorophyll (mg g^{-1} f.w.)	0.996±0.018	0.737±0.014***	1.461±0.044	1.000±0.075**	1.500±0.008	0.777±0.012***
IAA Oxidase (mg IAA degraded min^{-1} mg protein^{-1})	0.011±0.000	0.013±0.000***	0.016±0.000	0.019±0.000***	0.013±0.000	0.015±0.000***
Roots						
Protein (mg g^{-1} f.w.)	4.121±0.013	1.730±0.010***	6.196±0.013	3.282±0.007***	7.083±0.007	4.926±0.008***
Phenol (mg g^{-1} f.w.)	8.596±0.013	9.084±0.013***	10.072±0.021	11.824±0.020***	10.292±0.023	13.612±0.219***
Thiol (mg g^{-1} f.w.)	3.282±0.036	5.515±0.037***	4.313±0.012	5.906±0.026***	4.739±0.015	6.913±0.033***
Proline (mg g^{-1} f.w.)	0.485±0.006	0.719±0.020***	0.723±0.039	0.753±0.017ns	0.846±0.010	1.302±0.091**
Ascorbic Acid (mg g^{-1} f.w.)	0.296±0.006	0.428±0.001***	0.333±0.007	0.538±0.003***	0.550±0.001	0.733±0.003***
α-tocopherol (mg g^{-1} f.w.)	6.93±0.067	10.41±0.024***	10.77±0.035	13.43±0.015***	12.15±0.025	14.55±0.030***
IAA Oxidase (mg IAA degraded min^{-1} mg protein^{-1})	0.009±0.000	0.013±0.000***	0.014±0.000	0.022±0.000***	0.017±0.000	0.008±0.000***

[P_s: Photosynthetic rate; g_s: Stomatal conductance; Ci: Internal CO_2; WUE: Water use efficiency; F_0: Initial fluorescence; F_m: Maximum fluorescence; F_v: Variable fluorescence; F_v/F_m: PS II quantum efficiency]

not significant for roots as per the results of ANOVAR (Table 2). In leaves, proline content increased significantly under s-UV-B treatment at all ages. Maximum increment was observed at 40 DAT (66.4%, P <0.001). Treated roots also depicted a similar trend though increase in the amount of proline at 70 DAT was not significant. Proline content was higher in leaves compared to the roots under control conditions at all ages but higher in s-UV-B treated roots at the final sampling age (Table 1). Proline content in both leaves

Table 2 — Repeated Measures ANOVA (ANOVAR) test to determine the effects of the UV-B (T), plant age (A) and their interactions on the growth- and physiological characteristics and metabolites in leaves and roots of *Withania somnifera*.

	A	T	A×T
Growth parameters			
Total biomass	1543***	513.3***	47.1***
Root length	643.2***	98.4***	0.586ns
Shoot length	2348***	71.2***	44.3***
Plant height	7274***	16.7**	90.1***
No. of leaves	2077***	303.3***	96.3***
Leaf area	4322***	22.8**	6.0*
Root girth	781.6***	219.0***	66.5***
No. of flowers	1012***	114.4***	78.6***
No. of fruits	2863***	241.8***	295.2***
Physiological parameters			
Ps	860.9***	704.3***	0.353ns
Gs	189.4***	90.5***	12.9**
Ci	148.6***	515.8***	2.1ns
WUE	0.230ns	66.8***	6.4*
F0	40.1***	446.5***	0.174ns
Fm	43.3***	430.0***	10.5**
Fv	17.3***	668.7***	9.6**
Fv/Fm	1.7ns	632.0***	1.6ns
Leaves			
Protein	348000***	13410***	1880***
Phenol	35650***	590.0***	5.4*
Thiol	569.7***	797.1***	296.9***
Proline	1226***	170.9***	112.6***
Ascorbic Acid	2041***	2521***	172.4***
α-tocopherol	34150***	5594***	6.3*
Chlorophyll a	4832***	44060***	6569***
Chlorophyll b	39.2***	213.6***	1022***
Total chlorophyll	679.5***	232.8***	520.8***
IAA Oxidase	46.5***	490.0***	0.000ns
Roots			
Protein	96850***	172300***	140.1***
Phenol	795.1***	537.4***	164.6***
Thiol	532***	3004***	2.3ns
Proline	124.2***	56.8***	6.9*
Ascorbic Acid	5636***	1483***	45.7***
α-tocopherol	16240***	7596***	219.1***
IAA Oxidase	73.1***	16.1**	1245***

and roots was significantly affected by the individual factors of age and treatment as well as their interaction (Table 2).

To compare the means of various parameters between control and treated plants, Student's t-test was applied. The individual as well as interactive effects of s-UV-B treatment and plant age were computed through time via ANOVA with repeated measures (ANOVAR). All statistical analyses were performed using SPSS software v.16.

Results

Amongst the nonenzymatic antioxidants, both ascorbic acid (AA) and α-tocopherol depicted an increase under s-UV-B compared to the control plants. AA increased by 91.8%, 59.3%, and 86.6% in leaves and 44.6, 61.5 and 33.2% in roots at 40, 70 and 100 DAT, respectively. In both, control as well as treated organs, AA content was found to be higher in roots compared to the leaves (Table 1). Similar trend was depicted by α-tocopherol with both control and treated roots possessing it in higher concentrations compared to control and treated leaves. s-UV-B treatment enhanced its concentration in both the plant organs compared to their respective controls (Table 1). Minimum increment was recorded in leaves at 100 DAT (7.7%, P <0.001) and maximum in roots at 40 DAT (50.3%, P <0.001). The plant age, UV-B treatment and their interactions significantly affected both AA and α-tocopherol contents (Table 2).

Chlorophyll a, -b and hence, total chlorophyll content declined significantly at all sampling ages under s-UV-B treatment, the exception being a significant increment in chlorophyll b at the final sampling age (161.7%, P <0.001) (Table 1). ANOVAR results indicated the chlorophyll content to be significantly affected by plant age, s-UV-B treatment, and their interactive effects (Table 2). Enzymatic activity of IAAO increased significantly in treated leaves at all ages by 18.2, 18.8 and 15.4% at 40, 70 and 100 DAT, respectively (P <0.001). In roots, IAAO activity increased under treatment at first two sampling ages by 44.4 and 57.1% at 40 and 70 DAT, respectively while it declined at the last sampling age by ~53% (P <0.001) (Table 1). Also, IAAO activity was found to be higher in leaves compared to the roots under control conditions at the first two sampling ages while for the final sampling age the inverse was true. In UV-B treated organs, both leaves and roots depicted an almost equal IAAO activity at the initial sampling age; at 70 DAT it was higher in roots while at 100 DAT it was higher in leaves (Table 1). IAA oxidase activity was significantly affected in leaves due to plant age and s-UV-B treatment but not their interactions while in roots individual factors as well as their interactive effects significantly affected IAAO activity (Table 2).

Discussion

An overview of the effects of s-UV-B on the parameters studied here has been given in Fig. 1. A reduction in the total biomass of the test plant

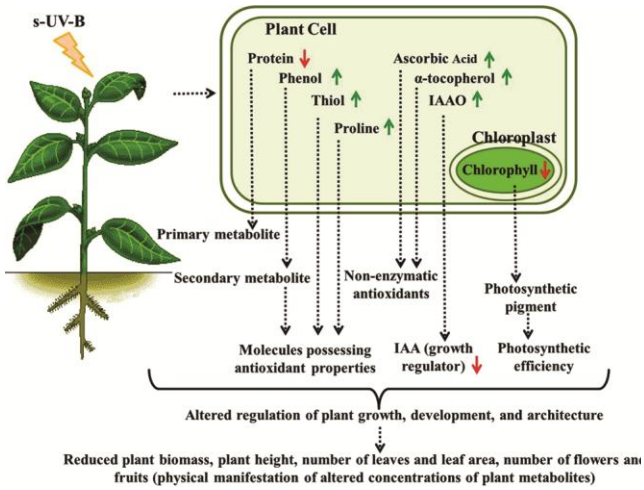

Fig. 1—An overview of the effect of s-UV-B on some plant metabolites/enzymes and consequent effects on plant growth and architecture

observed in the present study indicates diversion and utilization of photosynthates in the production of antioxidative defence compounds/enzymes and/or secondary metabolites[13]. Reduction in root length, shoot length, and consequently total plant height has been reported previously for medicinal plants such as *Acorus calamus*[14]. However, in our study, *W. somnifera* roots significantly increased under s-UV-B stress at all sampling ages. The plausible reason for this might be an increase in root peroxidases and consequent degradation of a natural phytohormone auxin, IAA[8]. This phenomenon has been suggested as a mechanism to induce elongation and gravitropism in roots[8] resulting in its increased length.

Our study revealed a reduction in shoot length under s-UV-B, the probable reason being the photooxidation of IAA and formation of growth-inhibiting photoproducts. The tips/growing shoot apices of plants are closest to the UV-B source. Since these are areas where IAA biosynthesis primarily occurs, sufficient amount of IAA is photodegraded by UV-B to alter plant growth and development, resulting in stunted plants. Photooxidation of IAA by UV-B has been observed UV-sensitive ecotype of *Spirodela punctata*[15] and has been proposed as a potent mechanism for UV-B-induced growth inhibition. Reduction in root girth under s-UV-B treatment at all ages was observed in our study. Other oxidative stresses, such as Cu toxicity in *Raphanus sativus*[16] have been known to produce similar results. Similar to our study, a reduction in number of leaves as well as leaf area has been observed in pea under supplemental UV-B radiation[17]. Decreased number of leaves might be due to reallocation of resources under stress conditions and reduction in leaf area is an adaptive strategy of plants to lower the absorbance of radiation which might be due to inhibition of cell division and cell expansion[18]. A reduction in flower number has been observed previously under UV-B stress in *Vigna radiata*[19]. Reduction in flower number could directly result in lower fruit setting.

The observed decrease of chlorophyll content in the present study can be directly correlated with a reduction in P_s which in turn is influenced by declined stomatal conductance (g_s). Similar results have been previously reported in mung bean[20]. s-UV-B was instrumental in inhibiting carbon assimilation, causing an increase in internal CO_2 concentration[20]. Water use efficiency also reduced under s-UV-B stress with one of the most plausible reasons being the decreased rate of photosynthesis. The results of the present study can be corroborated by previous similar studies on *C. forskohlii*[12]. Chlorophyll fluorescence measurements are reliable indicators of plant health. Increment in F_0 and reductions in F_m and F_v/F_m, under s-UV-B indicated a decline in energy transfer from chlorophyll a antenna complex to the reaction centres and/or inactivation of PS II reaction centres[21]. F_v/F_m ranging between 0.78 and 0.85 is indicative of healthy and nonstressed plants[22]. However, in our study, F_v/F_m values were below 0.78 at all sampling ages indicating detrimental effects to PS II activity and consequent reduction in photochemical efficiency.

Decreased protein content under UV-B radiation has been observed in recent studies in pea[17] and mung bean[20]. UV-B has the potency to cause protein degradation via destruction and/or modification of amino acid residues as well as through enhanced ROS production[23]. Altered DNA and RNA structures also hinder transcription and replication thereby decelerating protein synthesis under UV-B stress[23]. The decreased protein content in both the plant organs can be directly related with the reduced plant growth (reduced plant height, decreased in number of leaves and leaf area) affecting overall plant performance under stress. Increased phenolics under elevated UV-B prevents this radiation from reaching the more photosensitive targets (such as the photosynthetic apparatus), act as defence compounds against UV-B induced oxidative stress, and curtail UV-B induced damage to DNA[24]. Some of the phenolic compounds that are enhanced upon s-UV-B exposure, e.g. hydroxycinnamic acid derivatives, coumarins and their derivatives and naringenin, are instrumental in inhibiting plant growth[25].

Increased thiol content under enhanced UV-B has been previously observed in *A. calamus*[13]. Thiols, mainly in the form of (homo-)glutathione, may serve as antioxidants and play a role in protection against oxidative damage. The redox status of thiol compounds in both intra- and extracellular compartments is influential in determining protein structure and stability, enzyme activity regulation, and control of transcription[26] which in turn influences plant metabolic activity and hence plant performance. Increase in proline content might protect plant cells against UV-B induced peroxidative processes; proline accumulation under s-UV-B has been observed in *A. calamus* leaves[13]. Moreover, proline is also instrumental in regulating plant growth and development. Under stress conditions, proline accumulation influences energy-related pathways and carbon metabolism. Higher concentrations of proline have been known to negate cellular growth under normal osmotic conditions while also influencing ploidy level, vacuolation, and transcript accumulation pertaining to cell division and regulation of gene expression. Under stress conditions, proline might act as an energy source regulating plant metabolic activities relevant to stress adaptation[27].

Ascorbic acid (AA) is produced in higher concentrations by plants under varying stress conditions including UV-B[12]. Enhanced AA content under UV-B stress might be a strategy to alleviate ROS-induced oxidative stress. It also regulates plant growth and development by regulating cell division (via influencing cell cycle progression from G1 to S phase) and cell expansion[28]. α-Tocopherol is another important nonenzymatic antioxidant which, along with other antioxidants, protects plants against stress by acting as singlet oxygen quencher and stabilizing chloroplast membranes[29]. Higher levels of α-tocopherol, along with other antioxidants, were instrumental in improving the turf quality of Kentucky bluegrass preventing oxidative damage[29]. In *W. somnifera* leaves, along with some other antioxidants, α-tocopherol (vitamin E) content was detected under s-UV-B (indicating its enhanced concentration) via GC-MS analysis[30]. Besides their antioxidant function, tocopherols have been known to regulate gene expression, carbohydrate metabolism and photoassimilate transport[31] and hence plant growth and architecture.

Reduction in chlorophyll -a, -b, and hence, total chlorophyll content might be associated with the inhibition of chlorophyll synthesis via inhibition of aminolevulinic acid biosynthesis and/or via reduction in protochlorophyllides and breakdown of pigments and their precursors[32]. Decreased biosynthesis or degradation of chlorophyll is thought to be responsible for reduced chlorophyll content under supplemental UV-B radiation. Genetic regulation of chlorophyll biosynthesis and chlorophyll-binding proteins is also disturbed under s-UV-B, leading to reduction in chlorophyll content[32]. Moreover, excess ROS generated under UV-B are also instrumental in damaging photosynthetic apparatus. An unexpected increase in chlorophyll b content in our studies at final sampling age was observed which may be an acclimatization mechanism by Light-harvesting complex II (LHCII) (a protein-pigment complex) against elevated levels of UV-B[33]. Increment in IAAO activity observed in our study under s-UV-B is also reported in earlier studies on three *Ocimum* cultivars[34] under UV-B and can be directly correlated with the decrease in the shoot length of the test plant. However, the same cannot be held true for its root growth implicating other key players such as ROS, antioxidants, flavonoids and relative concentrations of loose and bound forms of IAA[8,35] to be involved in the process.

Conclusion

The results of the present study indicate that in *Withania somnifera*, s-UV-B causes an increment in secondary metabolites and antioxidants such as phenolics, thiol, proline, ascorbate and α-tocopherol which also regulate plant development (and consequently its architecture and performance), though at the cost of its growth and biomass. This indicates an attempt by the plant to counteract the adverse effects of UV-B. The obtained results further suggest that the s-UV-B treated plant parts can serve as a better source of secondary metabolites and antioxidants from the human health perspective. However, further studies using transgenic plants are required to elucidate further roles of these metabolites in plant growth and overall performance under stress conditions including s-UV-B.

Acknowledgement

The authors are thankful to the University Grants Commission (UGC), New Delhi, for financial assistance.

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