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Supplemental UV-B radiation induced changes in growth, pigments and antioxidant pool of bean (*Dolichos lablab*) under field conditions

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Abstract

Present study is conducted to evaluate the response of bean (*Dolichos lablab* cv . pusa early prolific) plants to supplemental UV-B (sUV-B: 280-315 nm: 7.2 kJ m^{-2} d^{-1}) radiation. UV-B caused alteration in biomass translocation pattern with more retention of biomass in below ground parts leading to an increment in root shoot ratio. Specific leaf area (SLA) which is the measure of leaf thinness, increased in plants under sUV-B exposure by 95.7 and 82.3% after 15 and 30 days after germination. Photosynthetic machinery of bean plants was the potential target of UV-B as photosynthetic rate was decreased by 88.6 % at 30 days after germination. sUV-B lead to the formation of reactive oxygen species thus generating oxidative stress. Stimulation of antioxidant defense system (enzymatic and non-enzymatic) was observed due to sUV-B radiation. Phenolic content decreased (34.7 and 18.6%) but protein showed varied response, increased initially (34%) thereafter declined (10.2%) under sUV-B radiation.

Key words

Dolichos lablab, UV-B radiation, Biomass, Specific leaf area (SLA), Photosynthetic system, Antioxidant defence system

Introduction

Solar electromagnetic radiation contains Ultraviolet-B (UV-B) in the range of 280-320 nm. Depletion of ozone (O_3) in the stratosphere has resulted in increased UV-B radiation reaching the earth's surface with serious implications on all the living organisms (McKenzie *et al.*, 2007). UV-B radiation has disproportionately large photobiological effects on plant genetic system (DNA), photosynthesis and membranes (Agrawal *et al.*, 2009). UV-B also generates reactive oxygen species (ROS) that result in inducting oxidative stress in plant cells (Kumari *et al.*, 2010). Concomitantly, transcripts of key enzymes of the antioxidative defense system, such as superoxide dismutase (SOD), peroxidases (POD) and catalase (CAT) are also induced upon exposure of UV-B irradiation (Singh *et al.*, 2009; Agrawal *et al.*, 2009). The majority of plant species including crops are negatively affected by UV-B radiation (Agrawal *et al.*, 2006). Exposure to supplemental UV-B (sUV-B) decreased plant height, leaf weight ratio (Johanson *et al.*, 1995), leaf numbers, leaf area, leaf area ratio (LAR), root shoot ratio (RSR), specific leaf weight (SLW) and plant dry weight while specific leaf area (SLA), number of nodes, auxiliary branching and leaf curling increased (Greenberg *et al.*, 1997; Furness *et al.*, 1999).

The photosynthetic apparatus is considered as one of the principal targets of UV-B radiation (Kumari et al., 2009). The sensitivity of species was partially explained by their ability to respond to UV-B by increasing the level of protective pigments or the leaf thickness (Caasi-Lit et al., 1997). Experimental studies of terrestrial plants to enhanced UV-B radiation often indicate enhanced leaf thickness (Johanson et al., 1995). Bean pods

have significant amounts of fiber and soluble fibre which help in lowering the blood cholesterol. Bean seeds are also rich in protein, complex carbohydrate, folate and iron (Osman *et al.,* 2007). The present study was conducted to evaluate the response of supplemental UV-B (sUV-B) radiation on various morphological, physiological and biochemical parameters including antioxidative defense system of bean plants (*Dolichos lablab* L. cv. Pusa Early Prolific) under field conditions.

Materials and Methods

Study site and raising of plant: Present study is performed at the Botanical Garden of the Banaras Hindu University, Varanasi (25°18' N latitude and 83°1' longitudes at an elevation of about 76 m above the mean sea level) situated in the Eastern Gangetic plain of India from February, 2007 to March, 2007. Soil of the study site was sandy loam in texture (sand 45, silt 28 and clay 27%, respectively). The mean monthly temperature varied from 14.3 to 28.1°C and total rainfall was 132.2 mm. Natural photosynthetically active radiation was 1547 µmol m^{-2} s^{-1} at mid day. *Dolichos lablab* L. (cv Pusa Early Prolific), a herbaceous annual plant is known as bean.

Seeds of bean were sown manually with 5 cm space in 24 pots of having diameter of 12.5 cm. The soil for pot filling was prepared after mixing the soil with farmyard manure in the ratio of 3: 1. Pots were watered regularly as and when required. After germination, plants were thinned to two plants per pot. Pots were randomly divided into two groups, *i.e.* (i) control, and (ii) sUV-B treated. For each treatment, twelve replicate pots were maintained.

Experimental design: The desired sUV-B radiation was provided by UV-B 313 lamps (Q-Panel, Inc. Cleveland, Ohio, USA). Banks of four lamps (120 cm long) fitted 30 cm apart on a mobile and adjustable steel frame were suspended above and perpendicular to the planted pots. Distance between the top of the plant canopy and lamps were maintained at 30 cm by adjusting the mobile frames. Plants were irradiated with supplemental dose of UV-B for 3 hr day^{-1} in the middle of the photoperiod. Each lamp was covered with 0.13 nm thick cellulose diacetate film (Cadillac Plastics, Baltimore, USA) which absorbed radiation emitted by lamps below 320 nm. For control, lamps were wrapped with polyster filters to exclude UV-B (318 nm). The UV-B irradiance under the lamps was measured at the top of the plant canopy by an ultraviolet intensity meter (UVP, Inc, San Gabriel, CA, USA). Plants beneath cellulose diacetate film received $UV\text{-}B_{BE}$ (ambient + 7.2 kJ m^{-2} d^{-1}) which mimicked 20% reduction in stratospheric O_3 at Varanasi during clear sky condition (Singh *et al.*, 2009).

Random sampling of bean plants was done in triplicate at 15 and 30 days after germination for biomass and biochemical analysis. Photosynthetic rate (Ps), stomatal conductance (g_s), transpiration (E) and internal CO_2 (Ci) were measured using portable photosynthetic system (Model LI- 6200, LI- COR, USA). The measurements were made on the third fully expanded leaf from the top of plant on cloud free days between 08.00 and 10.00 hr local time on three randomly selected plants using different pots. The amount of total chlorophyll and carotenoids were calculated by using the formulae developed by Maclachalan and Zalik (1963) and of Duxbury and Yentsch (1956), respectively. The absorption profile of flavonoid was estimated using the method proposed by Mirecki and Teramura (1984). The methods of Keller and Schwager (1977) were used for the extraction and determination of ascorbic acid. The total soluble protein content was estimated from fresh leaves of the plant by using the method given by Lowry *et al.* (1951). The phenol content of the supernatant was determined by Bray and Thorpe (1954). Superoxide dismutase (SOD) activity was measured by the method of Fridovich (1974). Ascorbate peroxidase and peroxidase enzyme activities were determined using the methods of Nakano and Asada (1987) and Britton and Mehley (1955), respectively. For total biomass, randomly sampled plants were oven dried at 80°C till a constant weight was achieved.

Results and Discussion

sUV-B radiation negatively affected the growth of the bean plants. Total biomass reduced by 61.6% at both the ages of observations (Table 1). Change in biomass accumulation is an important measure to assess sUV-B sensitivity, since this parameter reflects the cumulative effect of many small disruptions in plant function. UV-B radiation exclusion studies have also indicated that UV-B radiation reduced biomass accumulation in cucumber (Krizek *et al.*, 1997), spinach (Mishra and Agrawal, 2006) and mungbean (Agrawal *et al.*, 2006). Decrease in biomass observed in the present study may be caused by reduction in photosynthetic rate and enzymes activities as reported by Jordan *et al.* (1992). Root shoot ratio increased significantly by 103% at 30 days after germination under sUV-B treatment (Table 1). It remains unclear whether most UV-B induced changes in RSR resulted due to altered ontogeny or because of altered partitioning priorities. Increment in RSR values at later stage of sampling showed that more photosynthate was translocated to below ground parts to minimize the growth of aerial parts as a defense strategy. Agrawal *et al.* (2006), however, observed reduction in RSR of *Vigna radiata* under sUV-B radiation at all the ages of observation.

LAR showed significant increments of 60.5 and 57.4% whereas LWR reduced by 63.3 and 23.9%, respectively at 15 and 30 days after germination (Table 1). SLA increased significantly by 95.7 and 82.3 %, respectively in UV-B treated plants at 15 and 30 days after germination (Table 1). Results of two-way ANOVA showed that total biomass, RSR and LWR varied significantly with age, treatment and their interaction whereas SLA and LAR varied with individual factors of age and treatment (Table 2). SLA increased under sUV-B which led to more damage of mesophyll tissues thus significantly affecting the rate of photosynthesis.

Total chlorophyll and carotenoids contents were affected significantly after sUV-B exposure due to age, treatment and their interaction (Table 2). Total chlorophyll and chlorophyll a/b ratio decreased significantly in UV-B treated plants at both the ages of

Table - 1: Effect of sUV-B on total biomass, RSR, SLA, LAR, LWR, total chlorophyll, carotenoid, chlorophyll a/b and flavonoid of bean plants

Parameters	Plant age (DAG)	Control	sUV-B treated
Total biomass (g plant^{-1})	15	1.46±0.167	0.596±0.12** (-59.1)
	30	3.33±0.33	1.29±0.03*(-61.3)
RSR (g g^{-1})	15	0.365±0.13	0.356±0.029^{ns} (-2.47)
	30	0.133±0.005	0.270±0.016** (+103)
SLA (cm^2 g^{-1})	15	185.5±7.72	363.1± 6.69*** (+95.7)
	30	241.3± 7.23	439.9±5.5*** (+82.3)
LAR (cm^2 g^{-1})	15	107.7±4.39	172.9±5.66**(+60.5)
	30	90.23±2.01	142.0±1.21***(+57.4)
LWR (g g^{-1})	15	0.572±0.004	0.210±0.019*(-63.3)
	30	0.492±0.005	0.374±0.002*(-23.9)
Total chlorophyll (mg g^{-1} dry wt.)	15	3.58±0.022	2.59±0.81** (-27.7)
	30	2.06±0.058	1.95±0.03^{ns}(-5.34)
Carotenoid (mg g^{-1} dry wt.)	15	0.945±0.005	0.864±0.005*** (-8.57)
	30	1.21±0.01	0.726±0.004*** (-40.0)
Chlorophyll a/b	15	2.71±0.043	2.16±0.019*** (-20.3)
	30	2.53±0.31	1.95±0.089* (-22.9)
Flavonoids (Absorbance 310 nm)	15	0.689±0.051	0.579±0.015*** (-15.9)
	30	0.651±0.075	0.915±0.307*** (+40.5)

Value in the parenthesis show percent change; +/- denotes increase or decrease, Values are mean ± SE, RSR = Root shoot ratio, SLA = Specific leaf area, LAR = Leaf area ratio, LWR = Leaf weight ratio, DAG = Days after germination

Table - 2: F-ratios and level significance of two-way ANOVA for different parameters of bean plants exposed with sUV-B radiation

Parameters	Age	Treatment	Age x treatment
Total biomass	43.5***	55.3***	8.99*
SLA	758.9***	94.2***	2.39ns
LWR	18.2**	594.3***	153.6***
LAR	241.6***	41.3***	3.22^{ns}
RSR	75.9***	12.3***	16.0**
Internal CO2	10.2*	807.3***	113.5***
Photosynthetic rate	45.4***	32458.7***	113.4***
Transpiration rate	3833.9***	10043.7***	537.9***
WUE	1.29^{ns}	3141.1***	1482.3***
Total chlorophyll	404.6***	107.5***	67.3***
Chlorophyll a/b	4.3^{ns}	36.1***	0.025^{ns}
Flavonoids	17.4**	4.76^{ns}	27.7**
Carotenoids	1716.7***	82.5***	868.4***
SOD	65.1***	2478.2***	204.6***
POD	463.9***	2252.3***	539.2***
APX	6038.4***	759.6***	108.3***
Ascorbic acid	386.0***	0.370^{ns}	26.4***
Phenol	1326.6***	415.7***	4.09^{ns}
Protein	644.1***	0.043^{ns}	22.9***

ns = not significant, *p<0.05, **p<0.01, ***p<0.001

observations (Table 1). Total chlorophyll content reduced by 27.8 and 5.3% at 15 and 30 days after germination, while carotenoids showed reductions of 8.6 and 40% at 15 and 30 days after germination (Table 1). Reduction in chlorophyll a/b ratio was observed at both the ages of observations (Table 1). A decrease in photosynthetic pigments has been evident during exposure to enhanced UV-B radiation in most of the crop species (Kakani *et al*., 2003; Agrawal and Rathore, 2007). Strid and Porra (1992) suggested that decline in chlorophyll level might be due to inhibition of cab gene, which codes for chlorophyll protein. The reduction in chlorophyll a/b ratio by sUV radiation might be due to greater reduction in chlorophyll a than chlorophyll b (He *et al*., 1993). Carotenoids play an important role against UV-B damage in higher plants. Carotenoids, the scavengers of singlet oxygen species formed during intense light, are involved in the light harvesting and protection of chlorophylls from photoxidative destruction. The reduction in carotenoid content may result either from inhibition of synthesis or from breakdown of the pigments or their precursors. Flavonoid content increased significantly at 30 days after germination (40.5%) (Table 2) after sUV-B treatment. Two-way ANOVA showed that flavonoid content varied significantly with age and due to the interaction between age and treatment (Table 2).

Two-way ANOVA showed that Ps, Ci, E, g_s and water use efficiency were also affected due to treatment, and interaction of age × treatment (Table 2). Significant reduction in Ps was observed in sUV-B treated plants at all the ages of observations. Photosynthetic rate reduced by 68.3 and 88.6% at 15 and 30 days after germination, respectively as compared to their respective controls (Fig. 1). The possible reason for reduction in photosynthetic rate in plants exposed to high levels of UV-B radiation was noticed due to altered stomatal functioning (Cechin *et al*., 2007) or to both stomatal functioning and density (Gitz *et al*., 2005). Photosynthetic rate may be reduced directly by the effect of UV-B radiation on photosynthetic enzymes (Kumari *et al*., 2009), or due to disruption of PSII reaction centers (Herrmann *et al*., 1997).

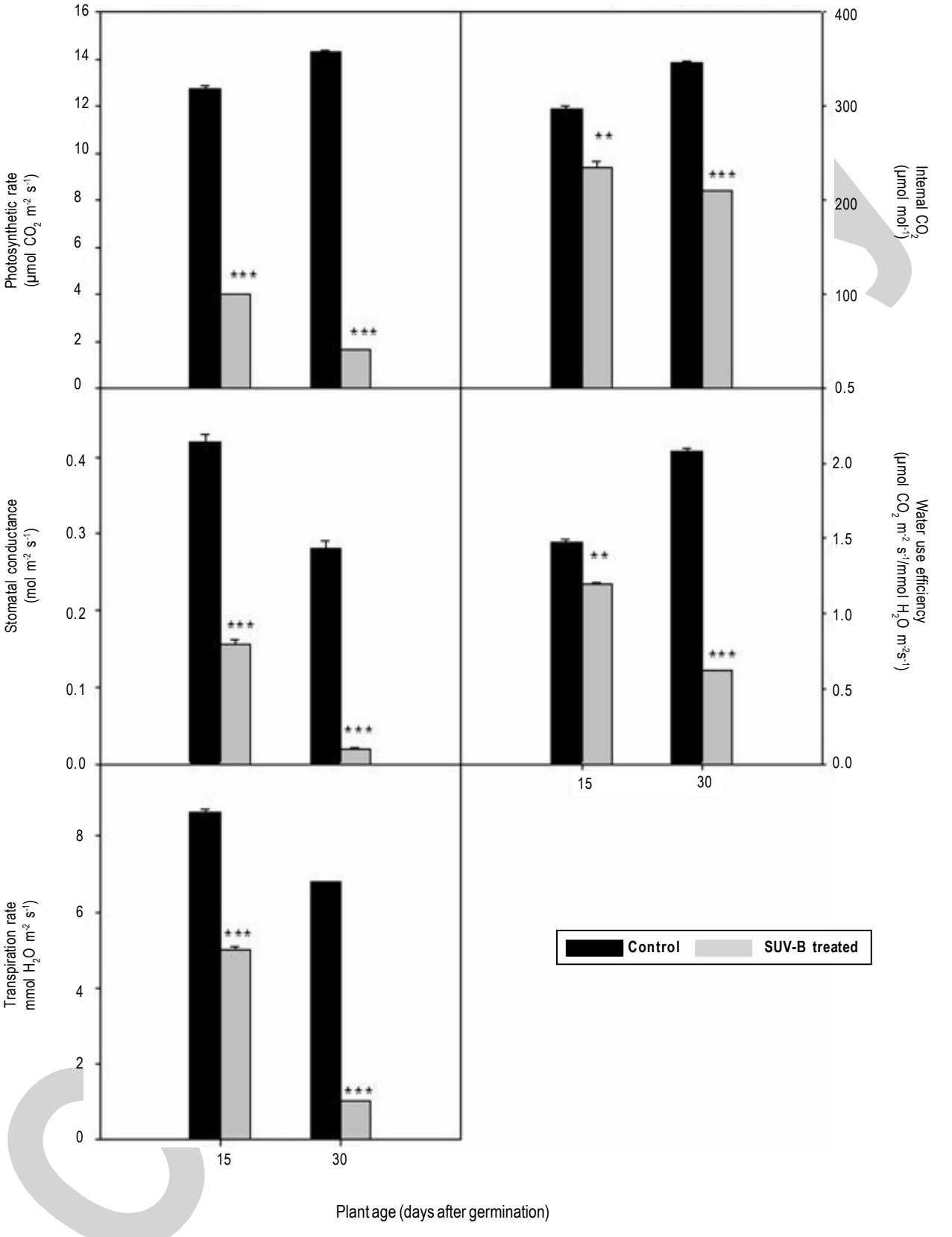

Fig. 1: Effects of sUV-B on photosynthetic rate, transpiration rate, stomatal conductance, internal CO_2, and water use efficiency of bean plants. Values are mean ± SE. Level of significance between control and sUV-B treated plants: ns, not significant, **p<0.01, ***p<0.001

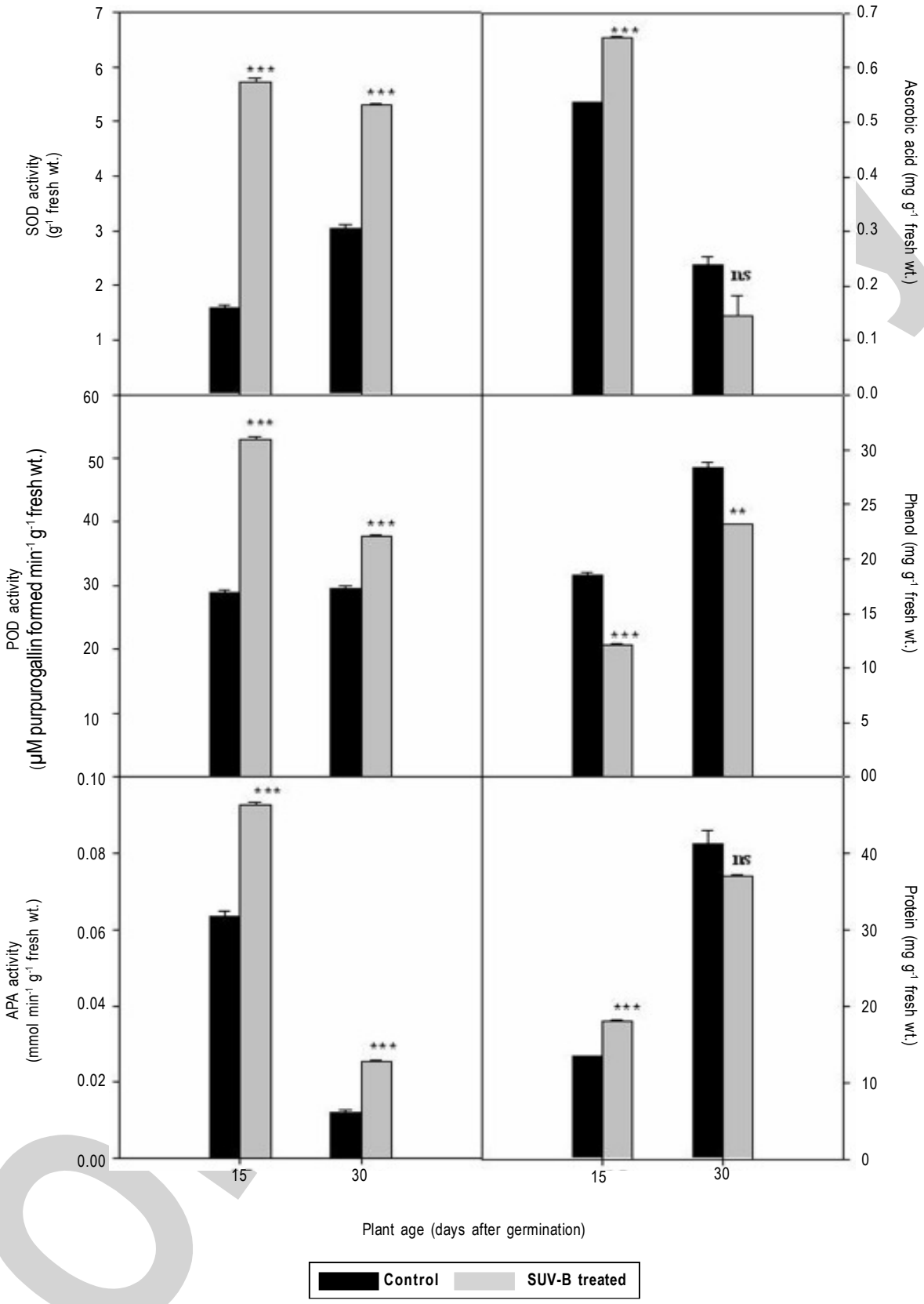

Fig. 2: Effects of sUV-B on POX, SOD and APX activities and ascorbic acid, phenol and protein contents of bean plants. Values are mean ±SE. Level of significance between control and sUV-B treated plants. ns= not significant, **p<0.01, ***p<0.001

Both internal CO_2 (Ci) and stomatal conductance (g_s) showed significant reductions of 20.8 and 39.4% and 62.8 and 22.8% at 15 and 30 days after germination, respectively (Fig.1). Transpiration rate (E) also declined significantly by 41.9 and 85% at 15 and 30 days after germination, respectively in sUV-B treated plants (Fig. 1). Values of water use efficiency (WUE) also reduced due to sUV-B treatment at both the ages of observations (Fig. 1). Internal CO_2 (Ci) decreased in test plants might be because of reduced stomatal conductance (Cechin *et al*., 2007). The transpiration rate was lower in exposed plants grown under sUV-B radiation and this inhibition was accompanied by a reduction in stomatal conductance. Cechin *et al.* (2007), observed that 35% decrement in E took place in sunflower plants after UV-B treatment. Plants' water use efficiency is an effective index for assessing the water use status of the plants. Qaderi *et al.* (2007) also reported reduction in water use efficiency (14.9%) of *Siliquas* sp. grown under 4.2 kJ m^{-2} d^{-1} of UV-B.

Protein and ascorbic acid contents varied significantly due to age and interaction between age and treatment, whereas phenol content varied significantly due to individual factors (Table 2). Protein content increased significantly by 34% at initial age (15 days after germination) and then declined by 10% at 30 days after germination (Fig. 2). sUV-B induced increment in total soluble protein content was also reported in rice (He *et al.*, 1994). It was argued that the increase in water soluble proteins was partially due to the stimulated production of several stress proteins participating in defense mechanism against UV-B stress. Phenol contents decreased with successive growth stage and showed reductions by 34.7 and 18.6% at 15 and 30 days after germination, respectively after sUV-B treatment as compared to control ones (Fig. 2).The increase in phenols may be attributed due to its increased synthesis from amino acids produced in plant cells during stress. Phenolics can protect DNA from UV-B induced damage (Mazza *et al*., 2000). Ascorbic acid showed an increment of 22% at 15 days after germination whereas reduction of 39% was observed at 30 days after germination (Fig. 2).

Results of two-way ANOVA showed that SOD, POD and APX activities varied significantly with age, treatment and also due to their interaction (Table 2). SOD and POD activities were induced significantly under sUV-B treatment in test plants at both the ages of observation, with maximum increment at 15 days after germination (257.1 and 82.7%, respectively). APX activity increased by 45.6 at 15 days after germination and by 167.4% at 30 days after germination (Fig. 2). Peroxidases in presence of phenols help to detoxify the increased amount of H_2O_2 in plants. Generation of UV-B induced reactive oxygen species (ROS) may trigger the response of antioxidative defense system. Enzymatic ROS scavenging mechanism in plants includes SOD, POX and APX. SOD acts as first line of defense against ROS. Yao and Liu (2006) reported decline in APX activity in *Acer mono maxim* leaves at high UV-B dose under field condition. Selvakumar (2008) observed increment in SOD in *Crotalaria juncea*, while Alexieva *et al.* (2001) reported increment of peroxidase activity in pea plants after UV-B exposure.

UV-B treatment negatively affected the bean plants under natural photosynthetically active radiation. Reduction in total biomass was accompanied by alteration in biomass translocation pattern. More biomass shift to the root portion lead to an increment in root shoot ratio. Reduction in leaf thickness under sUV-B leads to more damage to photosynthetic mesophyll tissue. Physiological responses showed that photosynthetic system was the potential target of sUV-B radiation. There was stimulation of enzymatic and non-enzymatic antioxidants under sUV-B treatment to cope up the oxidative stress generated by reactive oxygen species. Metabolites showed varied response under sUV-B radiation, while protein showed an increment as compared to control ones.

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References

Agrawal, S.B. and D. Rathore: 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**, 21-2 (2007).

Agrawal, S.B., D. Rathore and A. Singh: Combined effects of enhanced ultraviolet-B radiation and mineral nutrients on growth, biomass accumulation and yield characteristics of two cultivars of *Vigna radiata* L. *J. Environ. Biol.,* **27**, 55-60 (2006).

Agrawal, S.B., S. Singh and M. Agrawal: Ultraviolet-B induced changes in gene expression and antioxidants in plants. *Adv. Bot. Res.,* **52**, 47-86 (2009).

Alexieva, V., I. Sergiev, S. Mapelli and E. Karanov: The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ.,* **24**, 1337-1344, (2001).

Bray, H.G. and W.Y. Thorpe: Analysis of phenolic compounds of interest in metabolism. *In:* Methods of biochemical analysis (*Ed.*: D. Glick). Vol. 1, Interscience Pub. New York. pp. 27-54 (1954).

Britton, C. and A.C. Mehley: Assay of catalase and peroxidase. *In:* Method in enzymology (*Eds.:* S.P. Colowick and N.O. Kalpan). Vol 2, Academic Press Inc., New York. p. 764 (1955).

Cassi-Lit, M.M., J. Whitecross, M. Nayudu and G.J. Tanner: UV-B radiation induces differential leaf damage, ultra structural changes and accumulation of specific phenolic compounds in rice cultivars. *Aust. J. Plant Physiol.,* **24**, 261-274 (1997).

Cechin, I., T.F. Fumis and A.L. Dokkedal: Growth and physiological responses of sunflower plants exposed to ultraviolet-B radiation. *Cienc. Rur.,* **37**, 85-90 (2007).

Duxbury, A.C. and C.S. Yentsch: Plankton pigment monographs. *J. Marine Res.,* **15**, 19-101 (1956).

Fridovich, I.: Superoxide dismutase. *Adv. Enzymol.,* **41**, 35-97 (1974).

Furness N., M.K. Upadhyaya and D.P. Ormrod: Seedling growth and leaf surface morphological responses of three rangeland weeds to ultraviolet-B radiation. *Weed Sci.,* **47**, 427-434 (1999).

Gitz III, D.C., Z. Liu-Gitz, S.J. Britz and J.H. Sullivan: Ultraviolet-B effects on stomatal density, water-use efficiency, and stable carbon isotope discrimination in four glasshouse-grown soybean (*Glycine max*) cultivars. *Environ. Exp. Bot.,* **53**, 343-355 (2005).

Greenberg, B.M., M.I. Wilson, X.D. Huang, C.L. Duxbury, K.E. Gerhaddt and R.W. Gensemer: The effects of ultraviolet- B radiation on higher plants. *In: P*lants for environmental studies (Eds. W. Wang, J., Goursuch and J.S. Hughes). CRC Press, Boca Raton. pp. 1-35 (1997).

He, J., L.K. Huang, W.S. Chow, M.I. Whitecross and J.M. Anderson: Responses of rice and pea plants to hardening with low doses of ultraviolet-B radiation. *Aust. J. Plant Physiol.,* **21**, 563-574 (1994).

He, J., L.K. Huang, W.S. Chow, M.L. Whitecross and J.M. Anderson: Effects of supplementary ultraviolet-B radiation on rice and pea plants. *Aust. J. Plant Physiol.,* **20**, 129-142 (1993).

Herrmann, H, D.P. Hader, F. Ghetti: Inhibition of photosynthesis by solar radiation in *Dunaliella salina*: Relative efficiencies of UV-B, UV-A and PAR. *Plant Cell Environ*., **20**, 359-365 (1997).

Johanson, U., C. Gehrke, L.O. Bjorn, T.V. Callaghan and M. Sonesson: The effects of enhanced UV-B radiation on a subarctic health ecosystem. *Ambiol.,* **24**, 106-111 (1995).

Jordan, B.R., J. He, W.S. Chow and J.M. Anderson: Changes in mRNA levels and polypeptide subunit of ribulose 1,5-bisphosphate carboxylase in response to supplemental UV-B radiation. *Plant Cell Environ.,* **15**, 91-98 (1992).

Kakani, V.G., K.R. Reddy, D. Zhao and K. Sailaja: Field crop responses to ultraviolet-B radiation: A review. *Agric. For. Meteorol.,* **120**, 191-218 (2003).

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

Krizek, D.T., R.M. Mirecki and S.J. Britz: Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cucumber. *Physiol. Plant,* **100**, 886-893 (1997).

Kumari, R., S. Singh and S.B. Agrawal: Effects of supplemental UV-B radiation on growth and physiology of *Acoruc calamus* L. (Sweet flag). *Acta Biol. Crac. Ser. Bot.,* **51**, 19-27 (2009).

Kumari, R., S. Singh and S.B. Agrawal: Responses of ultraviolet-B induced antioxidant defense system in a medicinal plant *Acorus calamus* L. *J. Environ. Biol.,* **31**, 907-911 (2010).

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall: Protein measurement with folin phenol reagent. J. Biol. Chem., 193, 265-275 (1951).

Maclachlan, S. and S. Zalik: Plastid structure, chlorophyll concentration and free amino acid composition of a chlorophyll mutant of barley. *Can. J Bot.,* **41**, 1053-1062 (1963).

Mazza, C.A., H.E. Boccalandro, C.V. Giordano, D. Battista, A.L. Scopel and C.L. Ballaré: Functional significance and induction by solar radiation of ultraviolet-absorbing sunscreens in field-grown soybean crops. *Plant Physiol.,* **122**, 117-125 (2000).

McKenzie, R.L., P.J. Aucamp, A.F. Bais, L.O. Bjorn and M. Ilyas: Changes in biologically-active ultraviolet radiation reaching the earth's surface, *Photochem. Photobiol. Sci.,* **6**, 218-231 (2007).

Mirecki, R.M. and A.H. Teramura: 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 (1984).

Mishra, S. and S.B. Agrawal: Interactive effects between supplemental ultraviolet-B radiation and heavy metals on the growth and biochemical characteristics of *Spinacia oleracea*. *Braz. J. Plant Physiol.,* **18**, 307-314 (2006).

Nakano, Y. and K. Asada: Purification of ascorbate peroxidase in spinach chloroplast, its inactivation in ascorbate depleted medium and reactivation by monodehydroascorbate radical. *Plant Cell Physiol.,* **28**, 131-140 (1987).

Osman, M.A.: Effect of different processing methods, on nutrient composition, antinutritional factors and *in vivo* protein digestibility of *Dolichos lablab* bean [*Lablab purpuresus* (L) sweet]. *Pak. J. Nutri.*, **6**, 299-303 (2007).

Qaderi, M.M., D.M. Reid and E.C. Yeung: Morphological and physiological responses of canola (*Brassica napus*) siliquas and seeds to UVB and CO_2 under controlled environment conditions. *Environ. Exp. Bot.,* **60**, 428-437 (2007).

Selvakumar, V.: Ultraviolet-B radiation (280-315 nm) invoked antioxidant defense systems in *Vigna unguiculata* L. Walp and *Crotalaria juncea* L. *Photosynth.,* **46**, 98-106 (2008).

Singh, S., S. Mishra, R. Kumari and S.B. Agrawal: Response of ultraviolet-B and nickel on pigments, metabolites and antioxidants of *Pisum sativum* L. *J. Environ. Biol.,* **30**, 677-684 (2009).

Strid, A. and R.J. Porra: Alterations in pigment content in leaves of *Pisum sativum* after exposure to supplementary UV-B. *Plant Cell Physiol*., **33**, 1015-1023 (1992).

Yao, X. and Q. Liu: 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 (2006).