MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF KIDNEY BEANS (*PHASEOLUS VULGARIS* L. VAR. PUSA KOMAL) TO SUPPLEMENTAL UV-B RADIATION

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Abstract

Morphological and physiological responses of kidney beans (Phaseolus vulgaris L. var. Pusa komal) were investigated under supplemental UV-B (sUV-B radiation of 7.2 kJ m^{-2} d^{-1}) radiation. sUV-B radiation has altered the pattern of biomass allocation (change in RSR) and reduction in the biomass accumulation (43.99%) was obtained as compared to the control plant. sUV-B treated plants exhibit decrease in growth (stem, leaf, root etc.), physiology and increased protective mechanisms (thick leaves, increased flavonoid content etc.). Increase in chlorophyll content (90.40%) was obtained to compensate the decrease in rate of photosynthesis due to reduction in leaf area (55.23%). The study shows that Phaseolus vulgaris is UV-B sensitive plant species whose yield is negatively affected by sUV-B radiation.

Keywords: *Supplemental UV-B; Kidney beans; Growth; Yield; Fv/Fm*

Introduction

Depletion of ozone layer causes harmful effects on the earth surface. Plants and animals both get affected by the enhanced ultra violet B radiation because of the instability in the ecosystem caused by UV-B radiation. Animal and Plants have received a considerable attention in the assessment of the UV-B toxicity to terrestrial ecosystem (Caldwell et al., 2007). Effects on the animals and plants include adverse effects on the morphological, physiological and biochemical characters. The Montreal protocol and its amendment have given some relief against the ozone depleting substances and hence the concentration of ozone is expected to be recovered till 2050, if all the countries will implement such regulations.'

Material & Method

Experiment site

The field experiment was conducted from November 2011 to February 2012 at the Botanical Garden of Banaras Hindu University, Varanasi, Uttar Pradesh (25.8 ^{0}N, 83.1 ^{0}E; 76 m above sea level), which is on the eastern Gangetic plain of India. Soil at the study site was sandy loam (sand 45%, silt 28% and clay 27%) with a neutral pH (7.2–7.4). During the experiment, mean temperature ranged from 21.6 $^\circ$C to 29.8 $^\circ$C, total rainfall was 27.5 mm and relative humidity ranged from 67.51 % to 95.29 % .

Plant material

The kidney bean (*Phaseolus vulgaris* L. cv Pusa Komal), belonging to family fabaceae was selected as experimental plant. It is a herbaceous plant, having erect and bushy habit, and 20-60 cm tall. Bean is one of the winter seasonal food crops of the world. It is grown worldwide for its edible bean, popular both in dry and green bean. It is widely cultivated in suburban and rural areas of Varanasi, India.

Raising plant

Seeds of the plants were soaked in water

for 24 hours and allowed to germinate in the dark under controlled temperature & humidity for 2 days (24^{0}C and 35-40%).

Experimental design

Two experimental treatments were set as ambient and supplemental UV-B treated plants of *Phaseolus vulgaris* in 1x1 meter plot area. Ambient was taken as a control plant. The experimental design was randomly blocked with UV-B treatment. The nutrient treatments (N, P, K) was provided in the ratio of 20:80:40 (N : P : K). Nitrogen, phosphorus and potassium were supplemented in form of urea, single super phosphate and mureate of potash respectively.

UV-B exposure system

Supplemental UV-B was artificially provided by Q-panel UV-B 313 40W fluorescent lamps (Q panel Inc., Cleveland, OH, USA). Three lamps (120 cm long each) per bank were fitted at 30 cm distance apart from steel frame and were suspended perpendicular to the planted rows of each treatment. The lamps were covered by either 0.13 mm cellulose diacetate filter (transmission down to 280 nm) for sUV-B radiation or 0.13 mm polyester filters (absorbed radiation below 320 nm) for the control to provide ambient levels of UV-B. The control plants thus received only ambient levels of UV-B radiation. Lamps in frames were adjusted weekly to a distance of 50 cm to provide a mean supplemental UV-B radiation of 7.2 kJ m^{-2} d^{-1} (unweighted) to plant apices for 3 h daily over the middle of photoperiod (10:00 a.m. to 1:00 p.m.).

Plant sampling and Growth analysis

Plants were sampled randomly in triplicates from both the treatments control and treated at 60 days after germination. Monoliths of 15cm x15cm 10cm containing intact roots were carefully dug out and thoroughly washed under running tap water after placing them in sieve of 1 mm mesh size to remove soil particles adhering to the roots. Growth parameters such as number of leaves, plant height, leaf area, root shoot lengths and biomass were quantified. Leaf area was measured using portable leaf area meter (Model LI-3000, LI-COR, Inc., USA). For biomass determination, root and shoot portions were separated and oven dried at 80 ^{0}C till the weight become constant. The dry weights of plant component were measured and expressed as g $plant^{-1}$. Growth indices (RSR and SLW) were calculated by the method given by Hunt (1982).

Chlorophyll fluorescence analysis

Chlorophyll fluorescence Kinetics of attached leaves was measured using a Plant efficiency analyzer during the gas exchange measurements. Calculations were made from fluorescence parameters of the maximum quantum efficiency of PSII photochemistry (given by *F*v/*F*m). Measurements of *F*v/*F*m were made after dark adaptation for 30 minutes.

Photosynthetic and Non - photosynthetic parameters

Amount of chlorophyll and carotenoid was estimated, using the method given by Maclachlan and Zalik (1963) for chlorophyll and Duxbury and Yentsch (1956) for carotenoids. Fresh leaf of 0.1 g was taken from interveinal areas and crushed with 10 ml of 80% acetone and filtered with muslin cloth. Solution was centrifuged at 6000 rpm for 10 min. and supernatant was collected. Optical densities of the supernatant were taken at 480, 510, 645, & 668 nm (Model 167, Systronic Visicon, India) against blank (80% acetone). From given optical densities,

amount of chlorophyll a, b and carotenoids were calculated by using the following formulae:

Chlorophyll a (mg g^{-1}) = 12.3 D_{663}-0.86 D_{645} x V/1000 x w x d

Chlorophyll b (mg g^{-1}) = 19.6 D_{645}-3.6 D_{663} x V/1000 x w x d

Carotenoids (mg g^{-1}) = 7.6 D_{480}-1.49 D_{310} x V/1000 x w x d

Where, d= path length of light (cm); w= dry weight of sample; V= volume of extract; D= optical density.

Flavonoids

Flavonoids were assayed by the method of Mirecki and Teramura, (1984). For the estimation of flavonoids, 100 mg leaf sample was homogenized in 10 ml ethanol and acetic acid mixture (99:1 v/v). The solution was centrifuged at 8,000 rpm for 15 minutes. The absorbance of the extract was measured at 290, 300, 310, 320 nm on UV-Vis spectrophotometer (Model 166, Systronic India), and resulting absorbance was plotted. Flavonoids were measured as absorbance per mass of leaf (A mg^{-1} fresh leaf).

Statistical Analysis

All data was analyzed by using SPSS version 16. To check the significance between the mean values of control and sUV-B treated plants t-test was done.

Results

Foliar symptoms

UV-B exposure showed distinct effects in form of visible symptoms as cuping, curling on few mature leaves of kidney bean. Injury was recorded initially as brown and bronze spots on the leaf surface that later resulted in chlorosis, necrosis and desication of the leaves. Treated leaves were look thicker than the normal ones. Yellow or brown spots were initially present in the interveinal areas of the leaf which later converted into blackish brown spots covering more surface area, while control was showing normal leaves.

Morphological charecteristics

Supplemental UV-B reduced growth of the plants in terms of plant height, root length, internodal space, number of leaves, leaf area, number of pods , and weight of pods. The reduction in plant height, root length, inter nodal space, number of leaves, leaf area, number of pods , and weight of pods were 15.46%, 16.08% ,26.03%, 13.48%, 55.23%, 18.60%, and 54.69% respectively in sUV-B as compare to control (Table 2).

Table 1:. Effects of sUV-B on plant height, root length, no. of branches, Internodal space, no. of leaves, leaf area, no. of pods, weight of pods, no. of seeds, weight of seeds

Sl. No.	Parameters	Control	UV-B treated
1	Plant height (cm)	51.73±0.98	43.73±2.90***
2	Root length (cm)	18.9±3.52	15.86±2.03***
3	No. of branches	13.46±1.73	16.76±1.855***
4	Internodal space (cm)	23.43±0.49	17.33±1.15***
5	Number of leaves	29.43±0.66	25.46±3.28***
6	Leaf area (cm2)	912.5±2.65	408.8±9 14***
7	No. of pods	10.21±2.02	8.31±0.88***
8	Weight of pods (g)	30.46±3.58	13.8±2.04***
9	Number of seeds (Plant-1)	11.7±2.85	2.6±0.33***
10	Weight of Seeds (g plant-1)	5.17±0.075	0.84±0.02***

ns, not significant. * p <0.05; ** p<0.01; ***p<0.001 (significance level)

Table 2: Effects of supplemental UV-B on biomass (stem leaf shoot root), growth indices, (RSR, SLW) and total biomass.

Sl. No.	Parameters	Control	sUV-B treated
1	Stem weight (g)	3.35±0.28	1.79±0.31***
2	Leaf weight (g)	2.97± 0.51	0.72±0.005***
3	Shoot weight (g)	6.32±0.44	2.51±0.03***
4	Root weight (g)	2.32±0.3	1.97± 0.03***
5	RSR (gg-1)	0.37±0.01	0.75±0.02***
6	SLW (gg-2)	0.003±0.0006	0.002±0.00009***
7	Total Biomass (g)	9.57±0.38	5.36±0.04***

ns, not significant. * p <0.05; ** p<0.01; ***p<0.001 (significance level)

Table 3. Effects of sUV-B on Photosynthetic pigments and sUV-B screeing pigments

Sl.No.	Parameters	Control	sUV-B treated
1	Cholorophyll content (mg/g FW)	2.022±0.009	3.85±0.11***
2	Carotenoid (mg/g FW)	0.082±0.0006	0.104±0.001***
3	Flavanoids1	3.102±0.009	4.35±0.005***
4	Fv/Fm	0.737±0.008	0.634±0.04***

ns, not significant. * p <0.05; ** p<0.01; ***p<0.001 (significance level)

Biomass

Results of biomass measurement showed that sUV-B decreased total biomass (43.99%) (Table 1) as compare to control plants. Similarly, there was reduction in stem biomass (46.56%), leaf biomass (75.75%), shoot biomass (60.28%) and in root biomass(15.08%) as compare to their respective control (Table 1). Growth analysis showed significant reduction in root shoot ratio (RSR) by 102.7% and specific leaf weight (SLW) by 33.3%.

Physiological parameters

Chlorophyll fluoroscence measurements showed increase in initial fluoroscence (F_0) (14.9%) where as decrease was reported in maximum fluoroscence (Fm) (7.6%), variable fluoroscence (Fv) (18.65%) and quantum yield (Fv/Fm) (13.9%) under UV-B.

Photosynthetic and Non-photosynthetic pigments

A significant increment was reported in caritenoid content (26.8%), flavanoids (40.2%) and chlorophyll content (90.4%) in test plants under sUV-B irradiation (Table 2).

Discussion

Growth, biomass and yield decreases as a response of sUV-B and antioxidant capacity increases to detoxify the ROS generated by UV-B. Some visual symptoms also explain the effect of sUV-B and its severity. Cupping and curling of leaves are one of them. This may be due to Indol acetic acid (IAA) destruction on the upper surface of the leaves while the lower surface of leaf contains normal amount of IAA and thus shows normal growth, causing cuping and curling. The yellow brown spots on the

leaves were showing the destruction of chlorophyll pigment and the necrotic areas were due to phenolics which may have deposited beneath the epidermis of the leaf to detoxify UV-B radiation as a first defense mechanism. Appearance of chlorotic and necrotic patches was attributed to the decrease in leaf chlorophyll content (up to 40%) on exposure to UV-B (Strid and Porra, 1992).

Reduced leaf area reflects a specific photomorphogenetic response of plants to sUV-B, which is mediated by UV-B receptors (Ballare et al., 1995). Reduction in leaf expansion may be due to greater reduction or destruction of auxin (Ros and Tevini, 1995), which is an important growth hormone. Leaf growth can be limited by low rates of photosynthesis but in present study, it can not be the appropriate reason to reduce the leaf area because amount of total chlorophyll increased significantly. The correct reason here may be the hormonal imbalance resulting from the interaction between UV-B radiation and IAA metabolism (Ros and Tevini, 1995). Reductions in leaf area were attributed to reductions in cell size or reduction in cell number.

The reductions in leaf area of plants are due to smaller size of leaves, not due to reduction in number of leaves. Reduction in leaf area shows the adaptive mechanism of plants reduce the total absorption of the UV-B radiation and to sustain their lives under such harmful radiation (Krizek et al., 1997). Reductions of 33.9% and 35.4% in leaf area of *Vigna radiata* and *Vigna mungo* were observed when exposed with sUV-B as compared to UV-B radiation (Singh, 1996) and *Dolichos lablab* (Singh and Agrawal, 2011) in field condition.

Reductions in plant height are due to the reduction in inter nodal space which may be as a result of direct damage of macromolecules such as DNA (Britt, 1999) and proteins (Gerghand et al., 1999). However, some increment has also been reported for instance, in radish plant upon 20% sUV-B exposure under field condition (Nithia et al., 2005). There is no significant reduction in root length was found in this experiment, may be due to more photosynthate allocation towards them to enhance the absorption of more nutrients from soil, so that, detrimental effects of UV-B can be minimized by enhancing other defense mechanisms. A significant reduction in pod weight, not due to the reduction in pods number, revealed that there is alteration in photosynthate allocation and may be due to reduce protein content.

A reduction in biomass accumulation is often a reliable indication of plant's sensitivity to UV-B radiation, since it represents the cumulative effects of damage or disturbance in physiological function such as photosynthetic rate. The reduction in biomass under sUV-B exposure, may be attributed to the degrees in total leaf area, limiting the photosynthate production. In present study the reduction in photosynthetic rate is not significant due to high level of carotenoid and also due to more chlorophyll content the reduction in biomass may be due to alteration in photosynthate allocation. sUV-B also modifies the growth indices like RSR and SLW. A high RSR is characteristically associated with plants growing in nutrient deficient condition but in our case the nutrients are utilized for photosynthesis and used for the adaptive mechanism against sUV-B.

When high UV-B irradiances were used generally the pigments like chlorophyll decreases (Strid and Pora, 1992) and carotenoid increases but in this experiment both were found significantly. Chlorophyll was found more may be due to increased leaf thickness and to absorb more light to perform photosynthesis because of less leaf area. Carotenoid content was perhaps found more to quench the radicals as an adaptive mechanism.

Flavanoid content was also increased in the high fluence rate of UV-B (Mishra and Agrawal,2007). This increase in flavanoid concentration is due to a higher activity of the key enzyme PAL, which is a product of phenyl proponoid pathway. Flavanoid also protect the mesophyll cells by screening of the UV-B radiation, with resulted in a high concentration of the chlorophyll pigments thus there is no significant reduction has been reported in photosynthetic rate.

Conclusion

Present study showed that supplemental UV-B radiation has adversely affected the growth and yield of Kidney bean. Treated plants showed altered biomass allocation with higher RSR, thus reflecting more biomass allocation to roots. Number of seeds, weight of seeds and weight of pods were affected more negatively than the number of pods under sUV-B treatment. Photosynthetic pigments and UV-B screening pigments were also higher in the treated plants thereby providing protection to the underlying photosystem. Enzymatic and non-enzymatic parameters of kidney bean can be investigated to obtain more conclusive results on the impact of sUV-B radiation .

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