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PHYSIOLOGICAL RESPONSES OF FIELD GROWN *ZEA MAYS* L. PLANTS TO ENHANCED UV–B RADIATION

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AMBASHT N. K. and AGRAWAL M. *Physiological responses of fild grown* Zea mays *L. plants to enhanced UV–B radiation.* BIOTRONICS **24**, 15–23, 1995. Field grown corn (*Zea mays* L. cv VC_2) plants were treated at an enhanced level of ultraviolet–B radiation (predicted at 20% ozone depletion) from sowing to plant maturity. Photosynthesis rate was always lower in UV–B treated plants. Photosynthesis rate did not correspond to stomatal response. Total chlorophyll and carotenoid concentrations were higher in UV–B treated plants except at preflowering stage. Chlorophyll a : b ratio was lower in plants grown at enhanced level of UV–B till preflowering stage thereafter increased compared to the control. Anthocyanin concentration was higher initially but later reduced in UV–B treated plants. Absorbance profile of flavonoids showed higher values in plants treated at enhanced UV–B. Total plant biomass was higher for UV–B treated plants at all stages of growth compared to untreated control. Net primary productivity also showed a pattern similar to biomass accumulation. The study clearly indicates that corn is resistant to enhanced UV–B levels predicted at 20% ozone depletion under field conditions.

Key words: *Zea mays*; biomass; photosynthesis; flavonoid; stomatal conductance.

INTRODUCTION

During the last few decades, there has been increasing concern about the effects of several trace gases on the stratospheric ozone layer which shields the biosphere from the lethal UV radiation from the sun. Ozone layer abruptly cuts off the solar spectrum at approximately 280 nm and therefore reduction in the amount of stratospheric ozone will permit disproportionately large amount of UV radiation between 280–320 nm (UV–B) to penetrate to the earth surface (*11*). Significant reduction in stratospheric ozone on global basis has been reported over the years 1979–1991 (*18*). The levels of biologically effective UV–B radiation have also increased over large areas of earth.

Several studies have confirmed that UV–B radiation can deleteriously affect over all plant growth and fundamental physiological processes in a number of cultivated plant species (*22*). Caldwell (*11*) have reviewed the studies on morphological, physiological and biochemical responses of plant to enhanced UV

-B radiation. Net reduction in the crop yield has also been reported for majority of the plants (*17*).

Most of the work carried out on effects of enhanced UV-B on plants are from the mid and high latitude regions. There is dearth of data regarding the plant responses to UV-B in tropics under natural levels of photosynthetically active radiation (PAR). Tropics receive higher UV radiation flux compared to temperate and polar regions and the predicted further increase would lead substantially more total UV-B dose in the tropical region.

The present study was aimed to assess the response of *Zea mays* L. (corn) a C_4 plant to enhanced level of UV-B under natural levels of PAR. Studies with corn have shown variable sensitivity to enhanced irradiance of UV-B (*7, 23*).

MATERIAL AND METHODS

Study Area

Field experiments were performed from March to May at the Botanical Garden of Banaras Hindu University, Varanasi (25°18′ N latitude and 83°1′ E longitudes, at an elevation of about 76 meters above the mean sea level) situated in the Eastern Gangétic plain of India. The soil of the study site was sandy loam in texture (sand 45%, silt 28% and clay 27%) and neutral in reaction (7.0 to 7.2 pH). During the experiment, average temperature ranged from 30 to 40℃, relative humidity ranged from 20 to 65% and rainfall of 2.4 mm was recorded in the month of May. Photosynthetically active radiation level averaged 1244 μmol m^{-2} s^{-1}, at mid day.

Experimental Design

The field was prepared after amending farm yard manure. Corn seeds (*Zea mays* L. cultivar VC_2) were hand planted on 3rd March in rows spaced 0.3 m apart at a seedling density of 24 seeds m^{-2} in 6 plots of 1.5×1.5 m each. After germination, plants were thinned to maintain a distance of 20 cm in rows for uniformity in growth. Recommended dose of nitrogen, phosphorus and potassium (100 : 60 : 40) were added to all the plots at the age of twenty days. Watering was done when required to maintain the crops in a well watered condition. Plots were randomly divided into two treatments, i. e. with UV-B and without UV-B. For each treatment, three replicate plots were maintained.

UV-B Exposure System

Supplemental UV-B radiation was provided by Q panel UV-B 313 lamps (Q Panel Inc. Cleveland, Ohio, USA) by suspending them above and perpendicular to the planted rows. UV-B exposure system consisted of four vertical iron frames fitted in the ground and two adjustable iron frame on which UV-B exposure array was fitted. Each UV-B array has four UV-B lamps (1.2 m long) spaced 0.3 m apart. The lamps were covered with either presolarized 0.13 mm thick cellulose diacetate filter to provide supplemental UV-B (Transmission up to 290 nm) or covered with 0.13 mm polyester filters to exclude the supplemental

UV–B (Transmission up to 320 nm) for control. To minimize the effects of temperature and scattering of UV–B, a 6 cm wide aluminium reflectance strip was used above the lamp length. Filters were changed periodically to avoid aging effects on the spectral transmission of UV–B.

UV–B irradiance was continued for 3 hrs starting from 11 AM to 2 PM. UV –B irradiance at the top of the plant canopy was measured regularly by Black–Ray J–22/Long Wave Ultraviolet Intensity Meter (UVP, Inc, San Gabriel, CA., USA). This ultraviolet meter was calibrated against a spectro–power meter (Scientech Inc, Boulder, Colorado, USA). The exposure system was set to deliver 40% above ambient UV–B simulating a 20% reduction in stratospheric ozone at Varanasi, India during clear sky condition of summer *(15)*. In the equation of Green *et al.* *(15)*, it was assumed that ozone column thickness was 3.0 mm, the albedo was 0 and the scatter was 1.0.

Plant Sampling and Analysis

Plants were randomly sampled only from the 1.2 m^2 central section under the lamps for various observations. An average of three replicates was taken for each of the measured parameters. Harvests of plant material were made at 20, 40 and 60 days ages for biomass determination. Plants were thoroughly washed and then oven dried at 80℃ till constant weight was achieved. The dry weights of plants were measured. From the biomass value and plant age, the net primary productivity (g $plant^{-1}$ day^{-1}) was calculated. Photosynthetic rate and stomatal conductance were measured in the field at 20, 40, 50 and 60 days ages using LI–COR Model 6200 Portable Photosynthesis System (LI–COR, Lincoln, NE, USA). All physiological measurements were made on the third leaf below the apex of the stem.

Chlorophyll and carotenoid contents were quantified in acetone extract as described by Maclachlan and Zalik *(19)* and Duxbury and Yentsch *(12)*, respectively. For the estimation of absorbance profile of flavonoid, method described by Flint *et al.* *(14)* was followed. Anthocyanin content was estimated by following the method of Beggs and Wellmann *(4)*.

Data were analysed through analysis of variance (ANOVA) and student's t' test for assessing the significance of quantitative changes in different parameters due to UV–B treatment.

RESULTS

The results in (Table 1) summerize the effects of enhanced UV–B radiation on net photosynthesis (Ps) rates in fully expanded leaves at different stages of growth being representative of early vegetative, preflowering, flowering and grain fill stages, respectively. Analysis of variance test showed significant differences in photosynthesis rate due to treatment ($p<0.005$) and age ($p<0.005$) (Table 2). Ps rate was maximum at preflowering stage and then declined when the plants approached flowering and fruiting stages. Photosynthesis rate decreased by 30.7% in UV–B treated plants compared to the control at 60 days

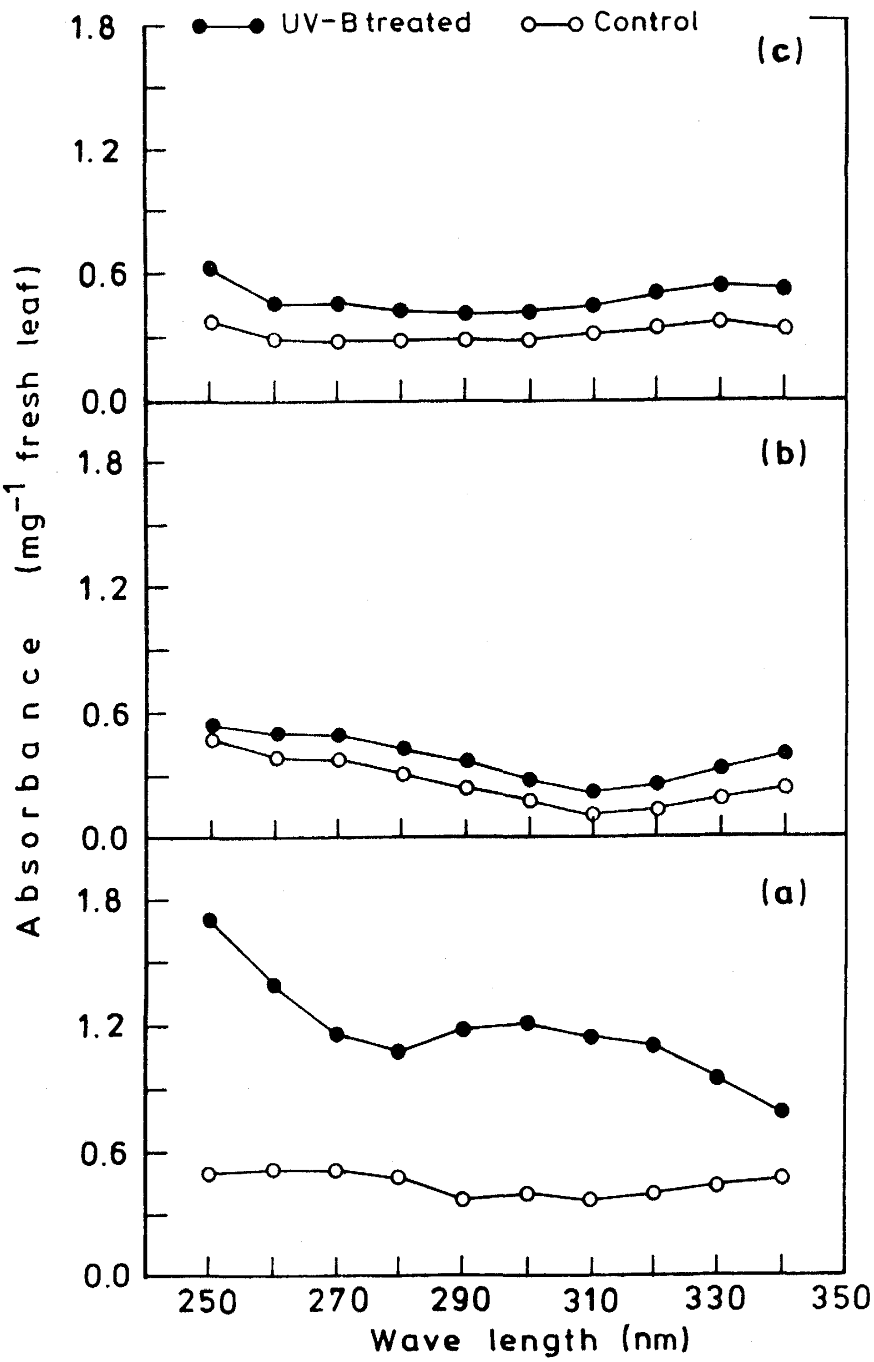

Fig. 1. Effect of UV-B radiation on absorbance profile of flavonoids at (a) 20, (b) 40 and (c) 60 days ages.

age.

Stomatal conductance was higher during preflowering and flowering stages compared to early vegetative growth and grain fill stages (Table 1). Stomatal conductance values were higher in UV-B treated plants compared to control except at the age of 40 days, the reduction being 6.4%.

Corn plants treated at enhanced UV-B radiation had higher chlorophyll and carotenoid concentrations per unit fresh weight than those of control plants at 20 and 60 days ages (Table 3). However, the trend was reverse at 40 days age. Chlorophyll a : b ratio was lower in UV-B treated plants at 20 and 40 days age but increased at 60 days. Anthocyanin concentration was higher in UV-B exposed plants at 20 days age but thereafter declined (Table 3). Absorbance profile of flavonoids showed higher values in UV-B treated plant at all stages (Fig 1), maximum increases being at early vegetative stage.

Biomass accumulation varied significantly in corn plants due to UV-B

Table 1. Effect of UV-B radiation on photosynthesis and stomatal conductance of *Zea mays* plant at different growth stages (Mean±1 SE)

Age (days)		Photosynthesis (μmol CO_2 m^{-2} s^{-1})	Stomatal Conductance (cm s^{-1})
20	C	22.73±1.39	0.779±0.012
	T	22.18±1.60	0.782±0.008
40	C	26.17±0.54	1.002±0.003
	T	23.20±0.82	0.956±0.001^{C}
50	C	14.90±0.19	1.278±0.004
	T	12.05±1.04	1.310±0.006
60	C	3.28±0.44	0.443±0.004
	T	4.58±0.04^{C}	0.506±0.001^{C}

Level of Significance: C: $p<0.001$
C=Control
T=UV-B Treated

Table 2. F ratios obtained in two way Analysis of Variance on physiological characters, biomass accumulation and chlorophyll concentration following UV-B treatments

Parameters	Treatment dF 1	Age	Treatment × Age Interaction
Photosynthesis	10.47**	228.05**	1.55^{NS}
Stomatal conductance	0.33^{NS}	237.81**	411.02**
Plant biomass	14.88**	5032.22**	5.57*
Total chlorophyll	617.86**	372.33**	233.14**

Level of Significance: NS: Not significant; $^{*}p<0.025$; $^{**}p<0.005$.

Table 3. Effect of UV-B radiation on pigment concentration of *Zea mays* at diffferent growth stages (The values represent mean±1 SE)

Age (days)		Total chlorophyll (mg g^{-1} fresh weight)	Chlorophyll a : b ratio	Carotenoid (mg g^{-1} fresh weight)	Anthocyanin (mg g^{-1} fresh weight)
20	C	2.40±0.1	3.34±0.4	0.85±0.005	0.030±0.001
	T	3.51±0.1[c]	2.87±0.02[b]	1.11±0.003[c]	0.038±0.002[a]
40	C	2.52±0.01	3.34±0.06	0.67±0.003	0.044±0.002
	T	2.39±0.01[c]	2.46±0.18[a]	0.59±0.04[NS]	0.033±0.039[NS]
60	C	1.67±0.01	1.65±0.01	0.47±0.02	0.024±0.001
	T	2.57±0.06[c]	1.76±0.03[c]	0.54±0.006[NS]	0.015±0.006[b]

Level of Significance: NS: Not significant; a: $p < 0.05$; b: $p < 0.01$; c: $p < 0.001$.
C=Control
T=UV-B Treated

Table 4. Effect of UV-B radiation on biomass accumulation and net primary productivity of *Zea mays* plant at different growth stages (The values represent mean±1 SE)

Age		Biomass (g $plant^{-1}$)	NPP (g $plant^{-1}$ day^{-1})
20	C	0.592±0.001	0.0295
	T	0.617±0.001[a]	0.0308
40	C	15.64±0.73	0.752
	T	17.20±0.62[NS]	0.829
60	C	85.33±0.66	3.48
	T	91.52±0.63[a]	3.71

Level of Significance: NS: Not significant; a: <0.05.
C=Control
T=UV-B Treated

treatment, age and their interactions ($p<0.005$) (Table 2). Biomass values were higher in UV-B treated plants at all harvests (Table 4), the increase being 4.2, 9.0 and 7.2%, respectively at 20, 40 and 60 days. Net primary productivity showed a similar pattern of change as that of biomass (Table 4).

DISCUSSION

The results indicate that supplemental UV-B radiation has led to significant reduction of photosynthetic rate. Allen *et al.* (*1*) have also shown a similar result. Enhanced UV-B radiation has been shown to affect photosynthesis directly by affecting primary photochemistry, electron transport, biochemical reactions of Calvin cycle, activity of enzymes as well as changes in the structure of chloroplast (*9, 16*). In the present study, the reduction in photosynthesis was not found associated with changes in stomatal conductance. A similar response for many rice varieties exposed to UV-B has been described (*23*).

The response of chlorophyll to UV-B radiation is not linear as this was higher in UV-B exposed plants at 20 and 60 days ages but lower at 40 days age compared to the control. Esser (*13*) has also reported an increase in chlorophyll concentration of spinach plants under high UV-B irradiance. Despite large effect of UV-B on photosynthesis rate no effect on chlorophyll concentration has been observed in soybean. In the present study also photosynthetic rate did not correlate with changes in chlorophyll concentration. Basiouny *et al.* (*3*), however, found that reductions in photosynthetic pigments were accompanied by the reduction in net CO_2 uptake rate in UV-B sensitive plants. Lower Chl $a:b$ ratio in UV-B treated plants up to preflowering stage was due to significant losses of both chlorophyll *a* and *b*. Strid *et al.* (*20*) have reported significant decline in Chl $a:b$ ratio in pea during UV-B treatment.

Interestingly the increase of chlorophyll and carotenoid contents were found directly related with the increase in appearance of pigments absorbing in the UV-B region. Flavonoids and anthocyanin levels were higher in UV-B treated plants at early vegetative stage with simultaneous increase in chlorophyll and carotenoids. The increase in flavonoids was not proportional to the length of UV-B treatment. Wellmann (*28*), however, showed that flavonol accumulation is linearly dependent on UV-B fluence. UV-B induced flavonoid synthesis has previously been reported in many plant species (*14, 21*). Flavonoids containing phenolic group can scavenge free radicals produced by UV-B (*8*) and thus protect the chlorophyll pigments from photooxidation. Anthocyanin level initially increased in UV-B treated plants but thereafter declined at successive growth stages. Wellmann (*27*) has, however, shown UV-B induced anthocyanin production in corn and wheat.

Exposure to supplemental UV-B radiation resulted in increase of biomass accumulation at all stages of growth (Table 4). Biggs and Kossuth (*7*) showed reductions in total dry weight and crop yield of corn exposed to enhanced level of UV-B. Other field studies have, however, shown increase in total dry weight of corn at higher levels of UV-B exposure (*2, 5, 6, 10*). Growth chamber and green house studies have also confirmed the tolerance of corn to increased levels of UV-B radiation (*3, 24, 25, 26*). The date presented here also indicate that corn is not sensitive to UV-B radiation with respect to biomass accumulation in the field condition.

In the present study, reduction in photosynthetic capacity was not found to be associated with changes in photosynthetic pigments. Reduction in photosynthesis rate did not even involve stomatal movement. UV-B absorbing pigments were, however, found to be directly correlated with changes in photosynthetic pigments. This study suggests that corn plant is tolerant to UV-B in field conditions in terms of biomass accumulation in plants. Physiological functions are, however, relatively sensitive at this exposure dose.

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