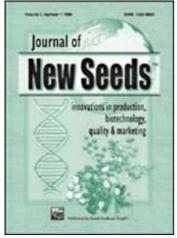
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Solar UV-B Exclusion Effects on Growth and Photosynthetic

Characteristics of Wheat and Pea

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Solar UV-B Exclusion Effects on Growth and Photosynthetic Characteristics of Wheat and Pea

M. Pal P. H. Zaidi S. R. Voleti A. Raj

ABSTRACT. Increased UV-B radiation on the earth's surface due to depletion of stratospheric ozone layer is one of the changes of current climate-change pattern. A field experiment was conducted to study the effects of solar ultraviolet-B (UV-B) radiation on growth parameters, net photosynthesis and biochemical characteristics of wheat (*Triticum aes-tivum*) and pea (*Pisum sativum*) crop species. Plants grown under ambient UV-B radiation were compared with those grown without UV-B by excluding ambient UV-B radiation. To exclude solar ambient UV-B, the sunlight was filtered through a polyester film that selectively absorbed UV-B. For ambient UV-B effects, plants were grown under polyvinyl chloride (PVC) filters that transmitted the complete light spectrum. The results indicate increased shoot length, leaf area, dry matter accumulation, leaf area ratio and specific leaf weight in plants of both the crops grown without UV-B compared with those grown under ambient UV-B. The effect of UV-B compared with those grown under ambient UV-B. The effect of UV-B exclusion was clearer in pea compared with that in wheat.

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Similarly, the rate of photosynthesis (measured as CO₂ exchange rate), chlorophyll content, nitrate reductase (NR) enzyme activity and sugar content were significantly higher in pea plants grown without UV-B radiation, while changes in wheat plants were marginal and insignificant. We conclude that monocot species may be less sensitive to increased solar UV-B due to ozone depletion compared with dicots. [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <docdelivery@haworthpress.com> Website: <http://www.HaworthPress.com> © 2006 by The Haworth Press, Inc. All rights reserved.]

KEYWORDS. Chlorophyll, growth, nitrate reductase activity, pea, photosynthesis, UV-B radiation, wheat

INTRODUCTION

In the past 50 years, the concentration of ozone has decreased by about 5%, mainly due to the release of anthropogenic pollutants, such as chlorofluorocarbons (Pyle, 1996). Consequently, a large proportion of the UV-B spectrum reaches the earth's surface with serious implications for all living organisms (Xiong and Day, 2001; Caldwell et al., 2003). There are reports on the impact of increased doses of UV-B radiation on crop plants (Singh, 1994). A high level of UV-B radiation has potentially deleterious effects on growth of plants, photosynthetic activity, flowering and crop yield (Teramura, 1983; Singh, 1994). Variability in crop responses to increased UV-B radiation has been observed due to differences in sensitivity of plant species (Teramura, 1983). There is also a tremendous natural variation in the daily effective UV-B radiation reaching the earth's surface (Ziska and Teramura, 1992). This variation occurs due to natural latitudinal gradient, atmospheric ozone column thickness, solar angle at different latitudes and elevation above sea level (Caldwell et al., 1980). Plants grown in low latitudes are exposed to higher flux of UV-radiation due to greater solar angle as compared with high latitude. India lies in a low ozone belt and receives more UV-B radiation compared with temperate higher latitudes (Mitra, 1991). Ambient solar UV-B affects plant growth and development in various ways (Pal et al., 1997; Krizek et al., 1997). Ruhland and Day (2000) observed a reduction in the vegetative growth of two vascular plant species of Antartica when grown with and without ambient UV-B.

Much of the early work concerning the effects of UV-B radiation on terrestrial plants was conducted indoors using growth chambers or greenhouses. By the 1990s, consensus was that many of these reports of UV-B effect were exaggerated and that extrapolation of these results to field responses was not appropriate (Caldwell and Flint, 1997). Since then, there has been emphasis on field studies either by supplementing natural UV-B or by lowering ambient UV-B by means of UV-B absorbing filters. Little is known about the response of crop plants to ambient solar UV-B radiation (Sharma et al., 1991; Searles et al. 1995, Pal et al., 1997; Krizek et al., 1997). In an earlier report, we found that the existing level of solar UV-B radiation during summer in the Delhi region was inhibitory to the growth and photosynthesis of mungbean (Pal et al., 1997). The level of solar UV-B radiation is different during summer and winter due to solar angle. Therefore, the affectivity of UV-B present in current solar radiation may vary during these two seasons. Thus, two winter crops, wheat and peas, were studied in the present investigation to evaluate their response to UV-B radiation during winter season in the Delhi region.

MATERIALS AND METHODS

Plant Material and Growth Conditions

A field experiment was conducted under natural sunlight at the Indian Agricultural Research Institute, New Delhi. Two crop species, viz., wheat (*Triticum aestivum* cv. HD 2285) and pea (*Pisum sativum* cv. JMR-10), were grown in the field under optimal growth conditions. The soil of the study site was sandy loam in texture and neutral in reaction (pH 7.0 to 7.4). The photosynthetically active radiation (PAR) averaged 955 μ mol m⁻² s⁻¹. Plots of 2 m × 2 m size were prepared using standard agronomic practices for growing the above crop species. The experiment was conducted in a randomized block design, and there were three replications each for control and treatments. Irrigation was given as and when required for optimal growth of the crops.

Solar UV-B Exclusion

In the first set of experiments, plants were grown under a 110 μ m thick polyester filter (UV-B-) that selectively absorbed UV-B (280-320 nm). In the second set, plants were grown under 100 μ m thick polythene sheets that transmitted the ambient UV-B radiation (UV-B+). The polyester film filtered out all the radiation below 310 nm and allowed only 23 percent of the 320 nm wavelength. The level of visible radiation through the polyester filter was about 85 percent of the unfiltered radiation at the ground level. Polythene film transmitted about 90 percent of the radiation in the UV-B region of solar spectrum. The visible radiation received through polythene film at the ground level was 89 percent of the ambient radiation. The spectral characteristics of the polyester and polythene filters were measured as described by Pal et al. (1997). The filters were replaced every two weeks as they became brittle due to solarization. Both polyester and polythene films were erected over a hut-shaped wooden frame fixed in north-south directions over the plots. This orientation ensured that solar radiation reached the plot only after passing through the filters as the sun moved from east to west. Bases of the wooden frames (0.35 m above ground) were left uncovered to ensure free airflow. Air temperature inside the wooden frames did not increase by more than 1°C above ambient.

UV-B Measurements

The level of ambient UV-B radiation on plant canopy was measured using UV-Biometer (Solar Light Co., PA, USA) and expressed as MED (minimum erythemal dose) and converted to μ W cm⁻² nm⁻¹ according to Srivastava et al. (1989). The UV-B radiation under the polythene film was recorded 20.71 μ W cm⁻² nm⁻¹ (2.18 MED), which was more than 90 percent of ambient UV-B radiation. Solar UV-B radiation under the polyester was negligible (0.09 MED).

Plant Growth Analysis

Plant growth measurements on shoot length, leaf area, leaf and stem dry weight were made at three stages: 30, 60 and 90 days after sowing (DAS). Ten plants of uniform size were selected for growth analysis from control and treatment plots each. Plant samples were separated into stem and leaves and their leaf area was determined using Leaf Area meter (Model LI-3100). For dry weight determination, samples were dried in a hot air oven at 70°C to a constant weight. Leaf area ratio (LAR) and specific leaf weight (SLW) were calculated using the formulae given by Gardner et al. (1988).

Rate of Photosynthesis and Biochemical Analysis

The photosynthetic rate (Pn) was measured in the topmost fully developed leaf with LI-COR portable photosynthesis system (Model, LI-6200, LI-COR, Inc., Lincoln, Nebraska, USA) between 1000 and 1130 hr when PAR ranged between 850 and 1200 μ mol m⁻² s⁻¹. Chlo-

Pal et al.

rophyll content was determined according to a non-maceration technique of Hiscox and Israelstam (1978) using dimethyl sulphoxide (DMSO). Nitrate reductase enzyme activity was estimated following the method of Klepper et al. (1971), as modified by Nair and Abrol (1973). Reducing and total sugars were determined using the Nelson's arsenomolybdate method (Nelson 1944). Non-reducing sugar content was estimated by subtracting the value of reducing sugar from total sugars.

Statistical Analyses

Analyses of variance of data were done as given by Panse and Sukhatme (1967). The critical difference (CD) values were calculated at 5% probability level.

RESULTS

Wheat and pea plants grown without solar UV-B (UV-B-) attained greater shoot length than plants grown under solar UV-B radiation (UV-B+). There was up to 7 percent greater shoot length due to exclusion of solar UV-B in wheat plants (Table 1). However, the increase in shoot length was more in pea compared with the 24 UV-B-free grown wheat plants. During the initial stages, an increase of 17-21 percent in shoot length of pea plants grown without UV-B was recorded, but the differences were smaller at later stages (7% at 90 DAS) (Table 2). Higher shoot length was accompanied by a significant increase in stem dry weight of UV-B-free pea plants (Table 2), but in the case of wheat, the gain in stem dry weight was slightly higher than that of ambient UV-B except at 60 DAS (Tables 1 and 2).

Leaf area of both wheat and pea plants grown without UV-B was higher than that for ambient UV-B. UV-B-free wheat plants possessed an average of 8 percent more leaf area than those grown under ambient UV-B (Table 1). In the case of pea, leaf area per plant increased by an average of 17 percent by excluding solar UV-B. Leaf dry weight of UV-B-free wheat plants was slightly higher than that under ambient UV-B at all stages (Table 1), but in the case of pea, there was a significant increase. Pea plants showed 14-23 percent more leaf dry weight as compared with ambient UV-B grown plants (Table 2).

Exclusion of solar UV-B did not affect significantly leaf area ratio (LAR) and specific leaf weight (SLW) of both wheat and pea. In the case of wheat, LAR of UV-B-free plants was marginally higher than that of ambient plants except at 60 DAS, where it showed a reduction of

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TABLE 1. Effect of solar UV-B exclusion (UV-B−) on growth parameters of wheat. All the values are means ±SE (n = 10). Figures in parenthesis indicate percent change between control (UV-B−) and ambient UV-B treated (UV-B+) plants.

Growth parameters			Days after sowing (DAS)	owing (DAS)		
	30	0	9	60	06	0
	UV-B+	UV-B-	UV-B+	UV-B-	UV-B+	UV-B-
Shoot length (cm plant ⁻¹)	17.5±0.68	18.9±0.57 (+8.0)	40.4±1.61	44.1±1.52* (+9.2)	54.7±1.66	57.2±1.48 (+4.5)
Leaf area (cm² plant ⁻¹)	68.3 ± 3.14	$74.7\pm3.88^{*}$ (+9.3)	215.67±12.07	234.12±12.48 (+8.5)	230.52±10.62	247.34 ± 11.85 (+7.0)
Leaf dry weight (g plant ⁻¹)	0.624 ± 0.019	0.661 ± 0.023 (+6.0)	1.460±0.049	1.580 ± 0.047 (+8.2)	1.554 ± 0.041	1.61 ± 0.054 (+3.8)
Stem dry weight (g plant $^{-1}$)	0.655±0.017	0.712±0.028 (+8.7)	2.680±0.072	0.083±3.020* (+12.7)	4.360±0.152	4.62±1.34 (+6.0)
Leaf area ratio (cm 2 g $^{-1}$)	52.63 ± 0.30	53.66±0.44 (+2.0)	52.31 ± 0.37	50.62 ± 0.27 (-3.2)	38.53±0.25	39.5 ± 0.32 (+2.5)
Specific leaf weight (mg cm $^{-2}$)	9.18±0.11	8.82±0.10 (-3.9)	6.74±0.09	6.70 ± 0.10 (-0.6)	6.71 ± 0.07	6.58 ± 0.09 (-1.9)
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* Levels of significance for difference between control and treated plants (P < 0.05).

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TABLE 2. Effect of solar UV-B exclusion (UV-B-) on growth parameters of pea plants. All the values are means ±SE (n = 10). Figures in parenthesis indicate percent change between control (UV-B-) and ambient UV-B treated (UV-B+) plants.

Growth parameters			Days after sowing (DAS)	owing (DAS)		
1	30		9	60	06	0
1	UV-B+	UV-B-	UV-B+	UV-B-	UV-B+	UV-B-
Shoot length (cm plant-1)	24.4±0.51	$29.5^{*}\pm0.55$ (+21.0)	72.0±1.61	$84.7^* \pm 1.54$ (+17.5)	108.2±2.22	115.7±2.41 (+7.0)
Leaf area (cm ² plant ⁻¹)	74.6±3.42	87.3*±4.07 (+17.0)	305.2±14.68	$366.5^* \pm 18.40$ (+20.0)	317.8±15.12	$364.3^* \pm 17.55$ (+14.5)
Leaf dry weight (g plant $^{-1}$)	0.385±0.011	$0.442^* \pm 0.014$ (+14.8)	1.743±4.92	$2.150^{*}\pm0.068$ (+23.0)	1.905±0.052	2.222*±0.061 (+16.6)
Stem dry weight (g plant ⁻¹)	0.468±0.013	$0.553^{\pm} \pm 0.017$ (+18.0)	3.186±0.090	3.720*±0.111 (+16.8)	6.770±0.207	$8.162^* \pm 0.223$ (+20.5)
Leaf area ratio (cm 2 g $^{-1}$)	86.37±2.86	88.40±2.55 (+2.4)	72.13±3.16	64.58 ± 2.12 (-10.5)	37.24±1.67	34.83 ± 1.48 (-6.5)
Specific leaf weight (mg cm $^{-2}$)	5.02±0.111	$\begin{array}{c} 5.14 \pm 0.098 \\ (+2.40) \end{array}$	5.74±0.177	5.83±0.132 (+1.60)	6.02±0.121	6.081 ± 0.140 (+1.00)
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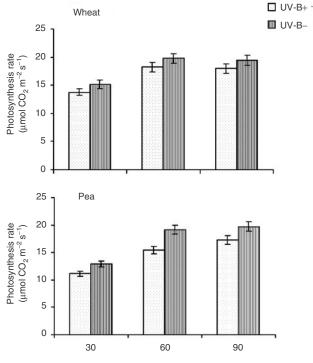
* Levels of significance for difference between control and treated plants (P < 0.05).

about 3 percent (Table 1). In UV-B-free pea plants, changes in LAR and SLW were almost opposite to those in wheat, where LAR was reduced significantly (at 60 DAS) and SLW was slightly higher than that for ambient grown plants at all stages (Table 2).

Leaf photosynthesis increased in both wheat and pea plants grown in the absence of solar UV-B. In wheat plants, UV-B exclusion had very little effect on net photosynthesis that increased by an average of 8 percent (Figure 1). In the case of pea, the increase in net photosynthesis was more and was recorded up to 24 percent higher (at 60 DAS) due to exclusion of UV-B (Figure 1).

No significant changes occurred in chlorophyll pigments of both the crop species due to solar UV-B exclusion. In wheat, chlorophyll 'b' content

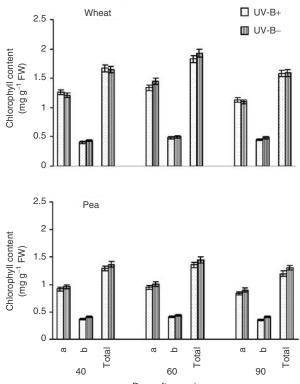
FIGURE 1. Changes in rate of photosynthesis of wheat and pea plants due to exclusion of solar UV-B radiation at different stages of growth. Error bar represent \pm SE of mean. UV-B + = ambient UV-B, UV-B = solar UV-B free.



Days after sowing

was marginally higher in UV-B-free grown plants at all the stages (Figure 2), but total chlorophyll content did not show an appreciable change at either stage. Chl. 'a' content was lower in UV-B-free grown wheat plants except at 60 DAS. In the case of pea, UV-B-free grown plants had higher Chl. 'a' and 'b' contents at all stages (Figure 2). The increase in Chl. 'a' due to UV-B exclusion was only slight at all stages but Chl. 'b' showed significant gain at 30 and 90 DAS. Total chlorophyll content in pea also showed only marginal gain as a result of solar UV-B exclusion.

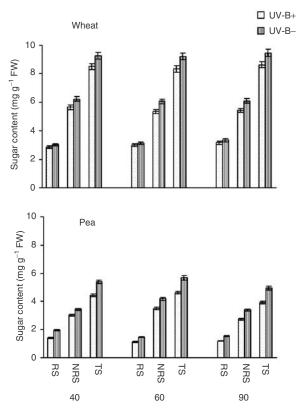
FIGURE 2. Changes in chlorophyll content of wheat and pea plants due to exclusion of solar UV-B radiation at different stages of growth. Error bar represent \pm SE of mean. UV-B+ = ambient UV-B, UV-B- = solar UV-B free. (a = chlorophyll 'a', b = chlorophyll 'b', total = total chlorophyll content)



Days after sowing

Reducing and non-reducing sugar contents of the leaves were also affected by the exclusion of solar UV-B. Both the sugar contents increased significantly in pea plants grown without UV-B, whereas in the case of wheat, the changes were relatively small (Figure 3). The highest increase in non-reducing sugar of wheat was 13 percent at 60 DAS in UV-B-free plants and no significant changes occurred in reducing sugars at either stage (Figure 3). The non-reducing sugar content of pea plants increased up to 24 percent increase in UV-B-free plants in com-

FIGURE 3. Changes in sugar content of wheat and pea plants due to exclusion of solar UV-B radiation at different stages of growth. Error bar represent \pm SE of mean. UV-B+ = ambient UV-B, UV-B- = solar UV-B free. (RS = reducing sugars, NRS = non-reducing sugars, and TS = total sugar content)

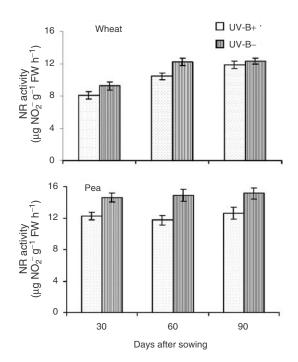


Days after sowing

parison with plants grown under ambient UV-B and reducing sugar content showed an increase of up to 39 percent (at 30 DAS). Changes in total sugar content were less in wheat plants compared with pea in UV-B-free treatment. Maximum increase in total sugars was observed at 90 DAS in pea plants (26%) due to exclusion of solar UV-B (Figure 3).

Solar UV-B exclusion also caused an increase in nitrate reductase (NR) enzyme activity in both wheat and pea plants. An appreciable increase in NR activity of UV-B-free grown wheat plants was observed at early stages (13 and 17 percent at 30 and 60 DAS, respectively), but the differences were not significant at maturity (4% at 90 DAS) (Figure 4). Pea plants grown without UV-B also showed significantly higher NR activity at all the stages and the highest increase was observed at 60 DAS (27%) (Figure 4).

FIGURE 4. Changes in nitrate reductase (NR) activity of wheat and pea plants due to exclusion of solar UV-B radiation at different stages of growth. Error bar represent \pm SE of mean. (UV-B + = ambient UV-B, UV-B - = solar UV-B free)



DISCUSSION

UV-B radiation causes reductions in plant height, leaf area, biomass accumulation and photosynthesis in various crop species suggesting that higher UV-B radiation is inhibitory to plant growth (Teramura, 1983; Singh, 1994). The present study showed that the current ambient UV-B radiation present in the Delhi region had a significant effect on the growth of wheat and pea plants. Exclusion of solar UV-B (UV-B-) caused up to 21% increase in the height of pea plants whereas in wheat there was only a marginal increase (up to 9%). The increase in plant height was accompanied by higher stem dry weight in UV-B-free grown plants of both the species. This suggests that the level of UV-B radiation present in solar radiation can potentially affect the plant growth and thus even small changes in ozone depletion may have important biological consequences. UV-B radiation higher than ambient UV-B radiation caused reduction in stem length and dry mass in pea (Mepsted et al., 1996). Similar effect of enhanced levels of UV-B radiation has been reported to affect plant growth of various other crop species like *Phaseolus mungo* (Singh, 1995), Vigna radiata (Pal et al., 1999), cucumber (Murali and Teramura, 1986) and cotton (Ambler et al., 1975).

The exclusion of solar UV-B radiation also affected the leaf area development as observed in this study. Up to a 20 percent increase in leaf area was observed in UV-B-free grown pea plants. Pal et al. (1997) reported a similar increase in leaf area of mungbean due to solar UV-B exclusion. Krizek et al. (1997) reported a 27 percent gain in stem length and a 35 percent increase in leaf area of cucumber plants grown in the absence of solar UV-B radiation. They also observed 34 and 55 percent greater accumulation in leaf and stem biomass, respectively, of cucumber. In the present study, UV-B-free grown pea plants gained 23 percent more leaf dry height than ambient-grown plants. However, UV-B-free grown wheat plants showed only small differences in leaf area and dry weight as compared with pea. This suggests that leaf orientation may be one of the reasons for wheat being less sensitive to ambient UV-B radiation than pea; wheat leaves are nearly vertical whereas pea leaves are horizontal in orientation. He et al. (1993) found that the effects of UV-B radiation were more severe in artificially constrained horizontal leaves than in the nearly vertical leaves of two rice cultivars. Gonzalez et al. (1998) also found that enhanced UV-B radiation caused a reduction in leaf area of pea.

The process of photosynthesis is considered to be sensitive to UV-B radiation. Such reductions in rate of photosynthesis have been attributed

to changes in photophosphorylation, electron transport and carbon assimilatory processes (Teramura, 1983). Similarly, Krause et al. (2003) have reported a reduction in net CO_2 uptake and photosystem I efficiency in tree seedlings when exposed to solar UV-B radiation. The net photosynthesis increased in *Populus* clones under sub-ambient (UV-B-) radiation (Schumaker et al., 1997). In this study, we found a higher rate of net photosynthesis in UV-B-free grown pea plants (up to 24%) against only a marginal increase (9.8%) in wheat plants grown under similar conditions. These results are consistent with our earlier findings in maize and mungbean (Pal et al. 1997). Zhao et al. (2004) did not find changes in growth and rate of photosynthesis at lower levels of UV-B radiation; but at higher levels they did find significant reductions in leaf area and rate of photosynthesis, and loss of rubisco activity. The increased plant growth and dry matter accumulation in UV-B-free grown plants may primarily be the result of increased photosynthesis.

The solar UV-B radiation also showed its impact on the process of nitrogen metabolism, which could be seen through changes in nitrate reductase enzyme activity. Dohler et al. (1987) showed that activity of nitrate reductase and other key enzymes of nitrogen metabolism were inhibited under enhanced UV-B radiation. In this study, the nitrate reductase activity increased by an average of 11 per cent in wheat and 13 percent in pea plants grown in the absence of solar UV-B radiation (UV-B-). The cause for the reduction in the nitrate reductase activity under enhanced UV-B might be the inhibition of ATP supply through a reduction in activity of photosystem II (Strid et al., 1990). Ming et al. (1998) have reported increased N content in the leaves of spring wheat due to enhanced UV-B treatment, which might be the result of inhibited N assimilation by the plants.

In both the crop species, rate of photosynthesis increased due to exclusion of solar UV-B, but chlorophyll content remained unaffected. This was in contrast to our earlier findings with mungbean and mustard grown under enhanced UV-B radiation where UV-B-irradiated plants showed reduced chlorophyll content along with lower photosynthesis rate (Pal et al., 1999). In this study, UV-B-free grown plants of both wheat and pea showed a marginal change in their total chlorophyll content in peach when grown under solar UV-B exclusion. Reduction in chlorophyll content along with lower rate of photosynthesis occurred in cotton under enhanced UV-B radiation (Kakani et al., 2004). We also found a higher level of reducing and non-reducing sugars in UV-B-free grown plants (UV-B-) of both the species. This could be the result of a higher

rate of photosynthesis in the absence of solar UV-B radiation. Increased levels of UV-B radiation have reportedly caused down-regulation of photosynthetic genes, leading to reduced levels of glucose in pea leaves (Mackernesi et al., 1997). Day et al. (1996) also reported that chlorophyll content decreased due to high UV-B irradiation in pea owing to reduction in expression of chlorophyll a/b binding proteins. Similar reductions in chlorophyll levels have been reported in rice (Ambasht and Agrawal, 1997).

These findings suggest that ambient solar UV-B present in solar spectrum in Delhi conditions affect plant growth, photosynthesis and other biochemical processes of crop plants, particularly of dicot species, because of their greater sensitivity to UV-B as compared with monocots. Further increases in the level of solar UV-B radiation due to depletion of stratosphere ozone level could be expected to be more inhibitory to dicot species than to monocots grown in this region.

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