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ARTICLE

UV-B Radiation: Anatomical, Physiological and Biochemical Changes in Glasshouse-Grown Ornamental Plants

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Three glasshouse-grown ornamental plants, viz. *Monstera deliciosa, Calathea lindeniana* and *Syngonium podoph* were treated with UV-B radiation at a level of 0.8 mW cm^{-2} for about 7 h daily for six days. Changes in leaf anatomy, phenolic compounds, photosynthetic pigments and chlorophyll fluorescence (F_v/F_m ratio) were studied. Leaves of the three plants showed changes in their inner structure. Phenolic compounds such as caumarin, flavonol, flavone and anthocyanin(s) were also accumulated in response to the UV-B treatment. Plants showed variability in accumulation of type of phenolic compounds in response to the treatment. Chlorophyll *a*, *b* and xanthophylls (violaxanthin, lutein, etc.) decreased to a greater extent while β-carotene remains unaffected. Photosynthetic efficiency of the plants changed to varying degrees in response to the UV-B treatment. We suggest that these anatomical and biochemical changes in plants take place in order to adapt/survive in the high UV-B environment.

Keywords: *Calathea lindeniana*, F_v/F_m ratio, *Monstera deliciosa*, phenolic compounds, photosynthetic pigments, *Syngonium podoph.*

Introduction

The stratospheric ozone layer is the primary layer which protects the living forms of the earth from UV radiation. It is believed that man-made atmospheric contaminants such as oxides of nitrogen (NO_x) and hydrogen (HO_x) released from high altitude aircrafts and spacecrafts and high concentration of chemical by-products of chloroflourocarbons result in the depletion of stratospheric ozone layer, which results in increased level of UV-B radiation reaching the earth's atmosphere. If the ozone layer depletion continues at this rate, there will be steady increase in the intensity of UV-B radiation on earth, which will cause serious ecological problems to all inhabitants of the earth. Plants are more prone to the increasing level of UV-B radiation as they require sunlight for synthesizing their food and therefore, cannot avoid the solar radiation.

The ultraviolet part of the spectrum is subdivided into three bands: UV-C (100–280 nm), UV-B (280–320 nm) and UV-A (320–400 nm; Caldwell, 1981). The shortest band UV-C is highly energetic and is effectively absorbed by the earth atmosphere. UV-B range of radiation is also highly energetic and is absorbed by the DNA and proteins in living organisms. The UV-B radiation is mainly attenuated by the ozone layer in the stratosphere. UV-A region of the spectrum is not so energetic and is also not attenuated by ozone. Thus depletion of ozone in stratosphere region is largely causing an increase in the UV-B range of radiation reaching the earth's atmosphere.

UV-B radiation affects the plants in various ways and shown to cause alterations in plant morphology, ultra-structure as well as in physiological and biochemical processes. Light harvesting system of the photosynthetic apparatus is one such part and undergoes relative changes in its constituent photosynthetic pigment, the chlorophyll and carotenoids (Warner and Caldwell, 1983). Leaves of the plants exposed to UV-B radiation are often characterized by a heavy waxy cuticle on the adaxial surface (Steinmulla and Tevini, 1985). The cuticle plays a role in protecting the leaf against UV-B damage by reflecting some of the incident radiation (Gauzman *et al.*, 1975). In addition, the adaxial epidermis of the leaf contains UV-B absorbing compounds which

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are effective in screening out much of the UV-B from more sensitive tissues (Flint *et al.*, 1985). Many phenolics are associated with increased UV-B resistance in plants, such compounds include flavonoids (Beggs *et al.*, 1985; Murali and Teramura 1985; Sharma *et al.*, 1997; 1998) and cuticular substances (Caldwell, 1981) and may prevent the damage to light-sensitive photosynthetic system by absorbing the UV-B radiation (Stapleton, 1992; He *et al.*, 1993; Middleton and Teramura 1993; Li *et al.*, 1993).

Ground-based and satellite instruments have measured decrease in the amount of stratospheric ozone over some part of Antartica. Up to 60% of the total overhead amount of ozone is depleted during August–December. This phenomenon is known as ozone hole. The size of the hole at present is as big as nine times the size of India. This depletion in stratospheric ozone is resulting in an increased level of UV-B radiation reaching the earth's atmosphere. Considering the research so far conducted specially with crop species, there is sufficient cause for concern about the consequences of stratospheric ozone depletion. Thus, there is a need to study how plants will respond to this continuously increasing UV-B radiation in the earth's atmosphere.

Materials and methods

Plant materials

Three different ornamental plants grown in pots in glasshouse were used for the study: 1, *Monstera deliciosa* (family Araceae); 2, *Calathea lindeniana* (family Marantaceae); 3, *Syngonium podoph* (family Araceae).

Growth conditions

Plants grown in earthen pots in glasshouse were used for the study. Being grown in the glasshouse they were unexposed to the UV-B radiation. The light intensity in glasshouse was approximately 150 μmol m^{-2} s^{-1}. Temperature and relative humidity during the study was 30 ± 2°C and 80% respectively.

UV-B treatment

The UV-B treatment (312 nm) of 0.8 mW cm^{-2} intensity was given to the plants using a UV-B light source (T-15) (Vilber-Lourmat, France). The UV-B radiation was measured using a UV-B (312 nm) radiometer (Vilber-Lourmat, France). Single leaf of the plant was kept horizontal to the UV-B source in order to expose the whole leaf uniformally with the UV-B radiation. Distance between the UV-B source and leaf surface was maintained to provide a specific dose (0.8 mW cm^{-2}) of the UV-B radiation. The UV-B treatment was given for about 7 h daily (10 : 00–17 : 00 h) for six days. The average level of solar UV-B radiation during the study occurring in Goa is shown in Table 1.

Anatomical studies

Sections of leaf through midrib were taken with the help of sharp blade. These sections were stained with the saffranine for 5 min in watch glass and mounted on a slide using glycerine as mountant and covered with cover slip. Slides were sealed with DPX to avoid any contamination. Photographs were taken using light microscope (Leica compound microscope) with a photographic attachment systems (Wild MP 32).

Extraction of phenolic compounds

Leaf tissue (1 g) was weighed and homogenised thoroughly in 10 ml of 80% methanol with 1% HCl using pestle and mortar. The final volume of the extract was made to 12.5 ml and was kept for dark extraction for 24 h. The extract was centrifuged at 4000 *g* for 20 min. The supernatant was collected and concentrated to 3 ml using water bath. The concentrated sample was used for spectrophotometric analysis of phenolic compounds using UV-B visible spectrophotometer (UV-240, Shimadzu) according to Sharma *et al.* (1997). The identification of flavonoids was carried out according to Swain (1976), based on their λ_{max}.

Table 1. Average level of solar UV-B radiation during the study (June–February 2000).

Time (h)	Level of UV-B radiation (mW cm^{-2})
10 : 00	0.326
13 : 00	0.602
17 : 00	0.240

Extraction of pigments

Leaf tissue (0.1 g) was weighed and ground thoroughly in 100% acetone containing a few crystals of BHT at 4°C. The final volume of the extract was made to 1.5 ml and centrifuged at 5000 rpm for 10 min at 4°C. The supernatant was collected in an eppendorf tube and were frozen at –70°C for HPLC analysis.

HPLC analysis of pigments

Identification and separation of pigments were carried out using HPLC (Spectraphysis, UK) with a C_{18} reverse phase column (ET 250 14 Nucleosil 100-5C 180 ODS). Spectraphysis SP ternary HPLC pump with SP 4270 integrator and spectra 100 variable wavelength detector. Before loading in HPLC column, the samples were filtered using 0.47 μm filters and 10 μl of the filtered samples was injected onto the HPLC column and separation was carried out using linear gradient of 0–100% ethyl acetate in acetonitrite : water (9 : 1 v/v) over 25 min at a flow rate of 1.2 ml min^{-1}. Peaks were detected at 445 nm and identified by using retention time of their standards (Sharma and Hall, 1996).

Chlorophyll fluorescence measurements

Chlorophyll fluorescence (F_v/F_m ratio) was measured using a pulse amplitude modulation fluorometer (PAM 101 & 102; Walz, Germany) according to Rodrigues *et al.* (2000). Prior to the measurements, leaves were dark-adapted for 10 min. The 1 cm^2 portion of the leaves was exposed to modulated light (4 μmol m^{-2} s^{-1}) to measure initial fluorescence (F_o) followed by the exposure of the leaves to a saturating pulse of white light (4000 μmol m^{-2} s^{-1}) to measure maximum fluorescence (F_m). Variable fluorescence (F_v) refer to F_m–F_o value.

Results and discussion

The effect of UV-B radiation on leaf of the different plants resulted in significant change in their anatomy. UV-B exposure to *M. deliciosa* resulted in the accumulation of reddish black compound within the cells (Figure 1D) which was observed to a lesser extent in control (Figure 1C). Further study with spectrophotometric analysis of the phenolic extract revealed that accumulated compound to be anthocyanin (Figure 4A).

UV-B exposure to *C. lindeniana* resulted in the formation of net-like structure in the intercellular space, probably scleroids, which was not seen in control plants (Figure 2D). The degradation of chloroplasts and synthesis of cuticle was also seen in *C. lindeniana* leaves exposed to UV-B radiation. The vascular tissue showed greater formation of sclerenchymatous tissue (Figure 2B).

In *S. podoph*, the UV-B treatment resulted in the synthesis of specialised honey comb-like structures grouped together below the epidermis (Figure 3C and D). Such structures were present in less number in control plant. It was also seen that UV-B exposure resulted in drying of the leaf to a large extent and distortion of cells.

The observed anatomical changes in leaves of various plants studied may represent the response of leaf to enhance UV-B radiation. The changes may act as defense or adaptation mechanism against enhanced UV-B radiation in order to prevent the penetration of UV-B radiation to more sensitive sites. The cuticle plays a role in protecting the leaf against UV-B damage (Gauzman *et al.*, 1975; Steinmulla and Tevini, 1985). It was reported by Robberecht and Caldwell *et al.* (1978) and Hahlbrock and Scheel (1989), and that cutin deposition along with flavonoid synthesis in upper epidermis could block transmittance of 95–98% of incoming UV-B radiation. However, plants studied in this work did not show large accumulation of cuticle.

Our results also show the large scale accumulation of reddish black compound (anthocyanin; a type of flavonoid) in the cells in *M. deliciosa* and to a lesser extent in *C. lindeniana* and *S. podoph*. This was in response to UV-B exposure in order to absorb the UV-B radiation, thereby preventing the UV-B damage to more sensitive sites such as DNA, protein, hormone, photosynthetic pigments, etc. Large increase in phenolic compounds seen in these plants may be due to less deposition of cutin in response to UV-B treatment.

The net-like structures seen in the intercellular space in UV-B-treated plants of *C. lindeniana* are probably

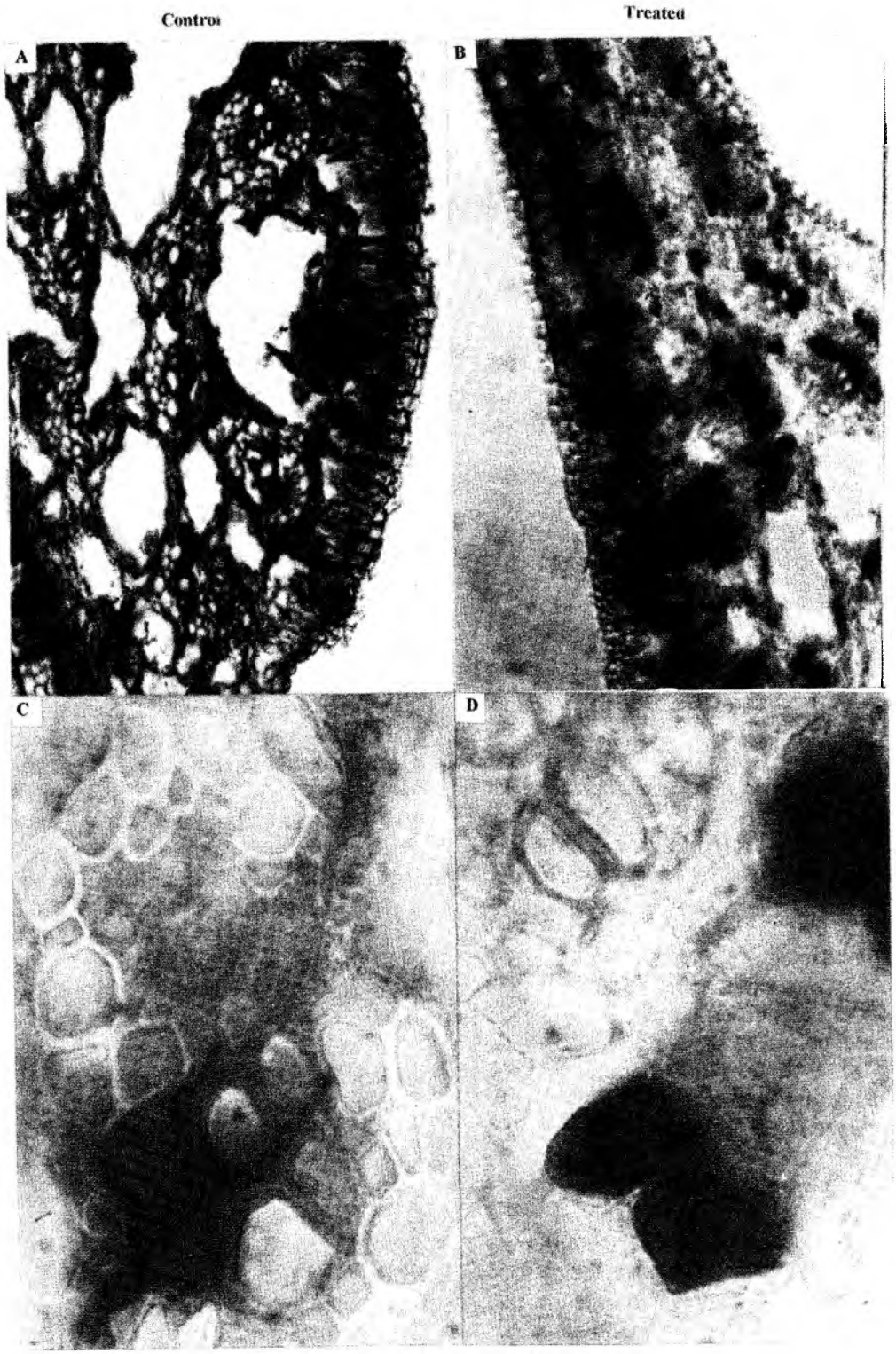

Figure 1. Effect of UV-B radiation (0.8 mW cm^{-2}) for six days on leaf anatomy of *Monstera deliciosa*. A, TS of control leaf (10X); B, TS of UV-B-treated leaf (10X); C, TS of control leaf (100X); D, TS of UV-B-treated leaf showing large accumulation of anthocyanin (100X).

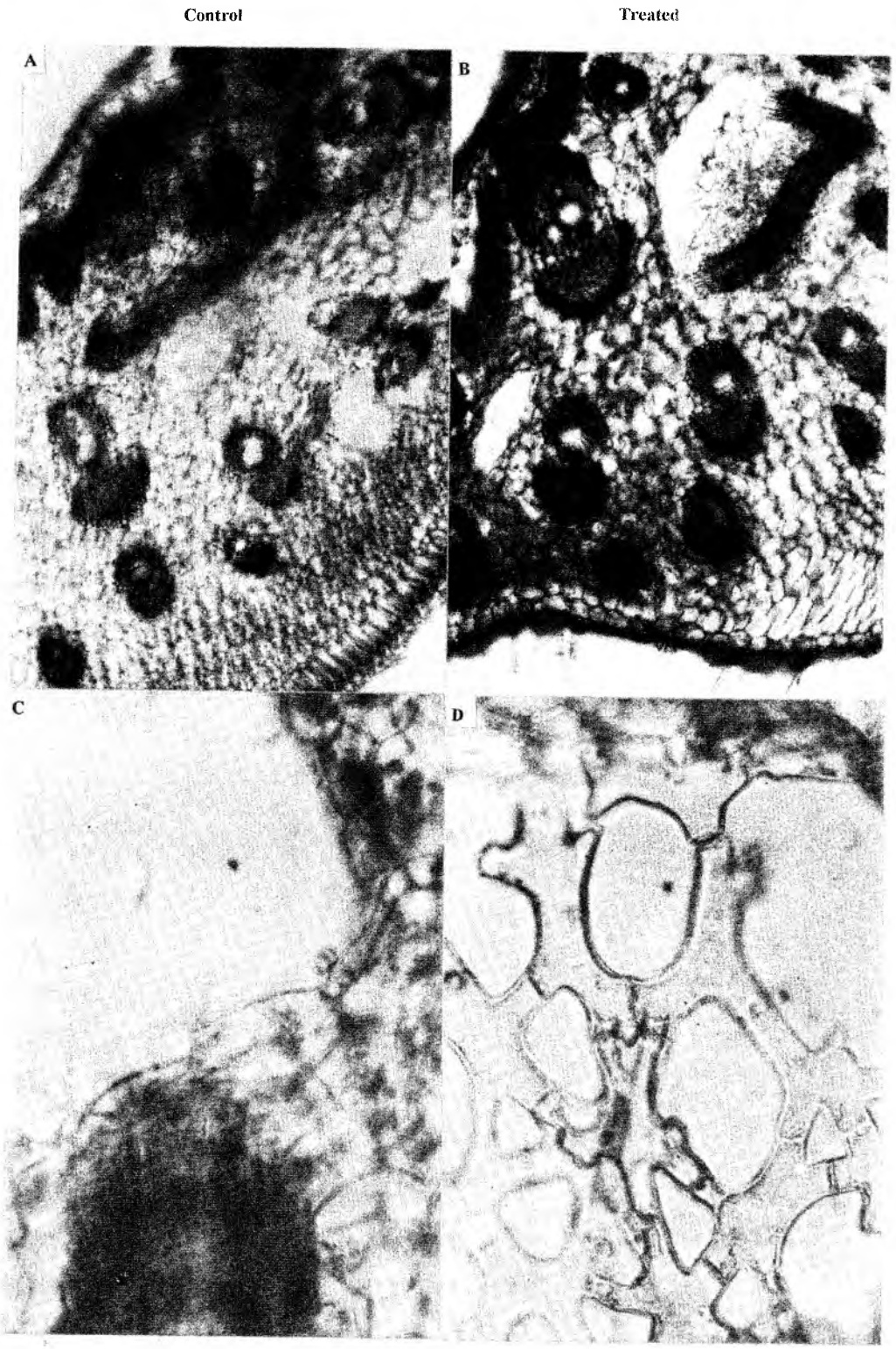

Figure 2. Effect of UV-B radiation (0.8 mW cm^{-2}) for six days on leaf anatomy of *Calathea lindeniana*. A, TS of control leaf (10X); B, TS of UV-B-treated leaf (10X); C, TS of control leaf (100X); D, TS of UV-B-treated leaf showing net-like structure in the inter cellular space (100X).

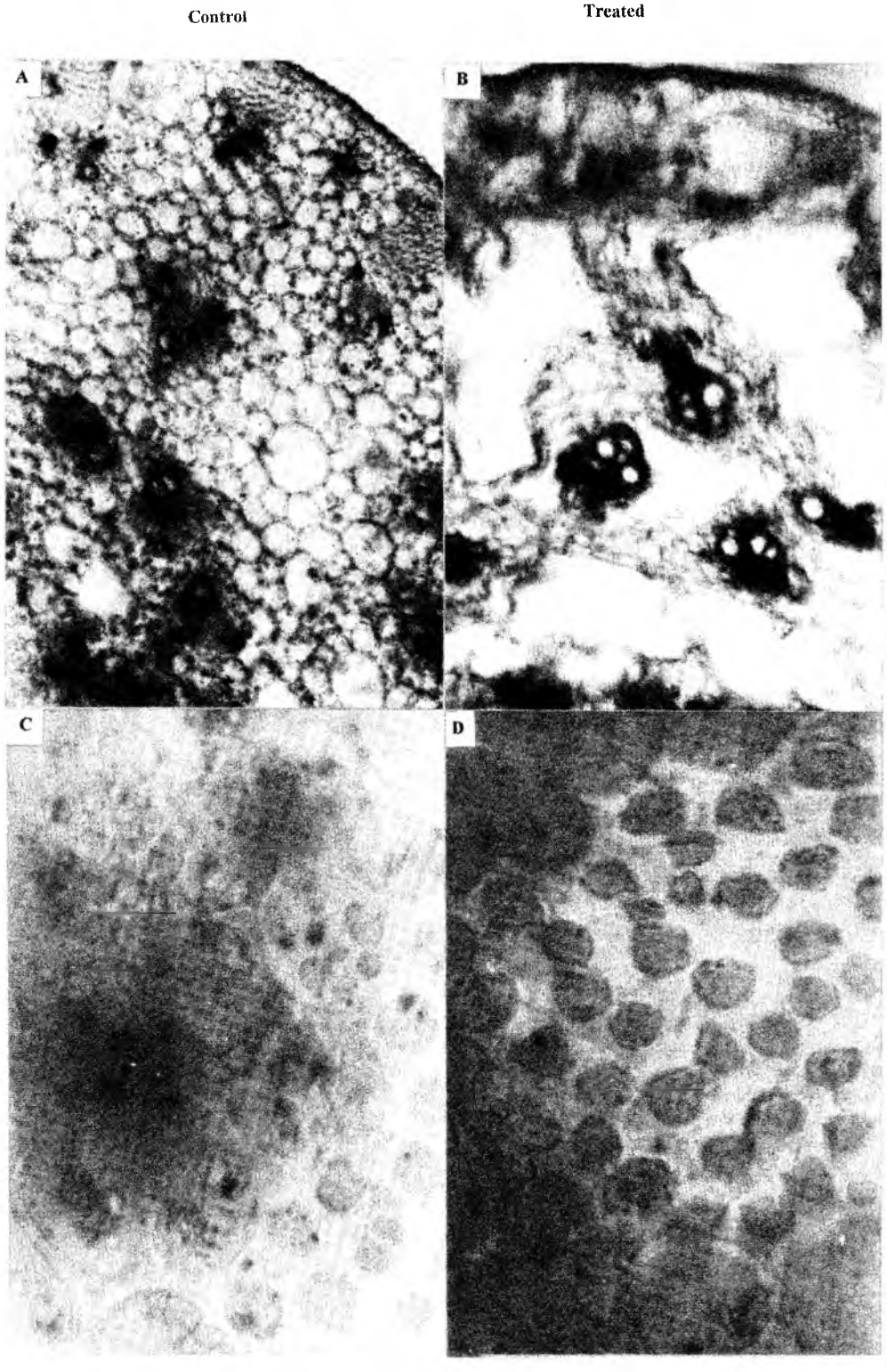

Figure 3. Effect of UV-B radiation (0.8 mW cm^{-2}) for 6 days on leaf anatomy of *Syngonium podoph.* A, TS of control leaf (10X); B, TS of UV-B-treated leaf (10X); C, TS of control leaf (100X); D, TS of UV-B-treated leaf showing honey comb-like structure (100X).

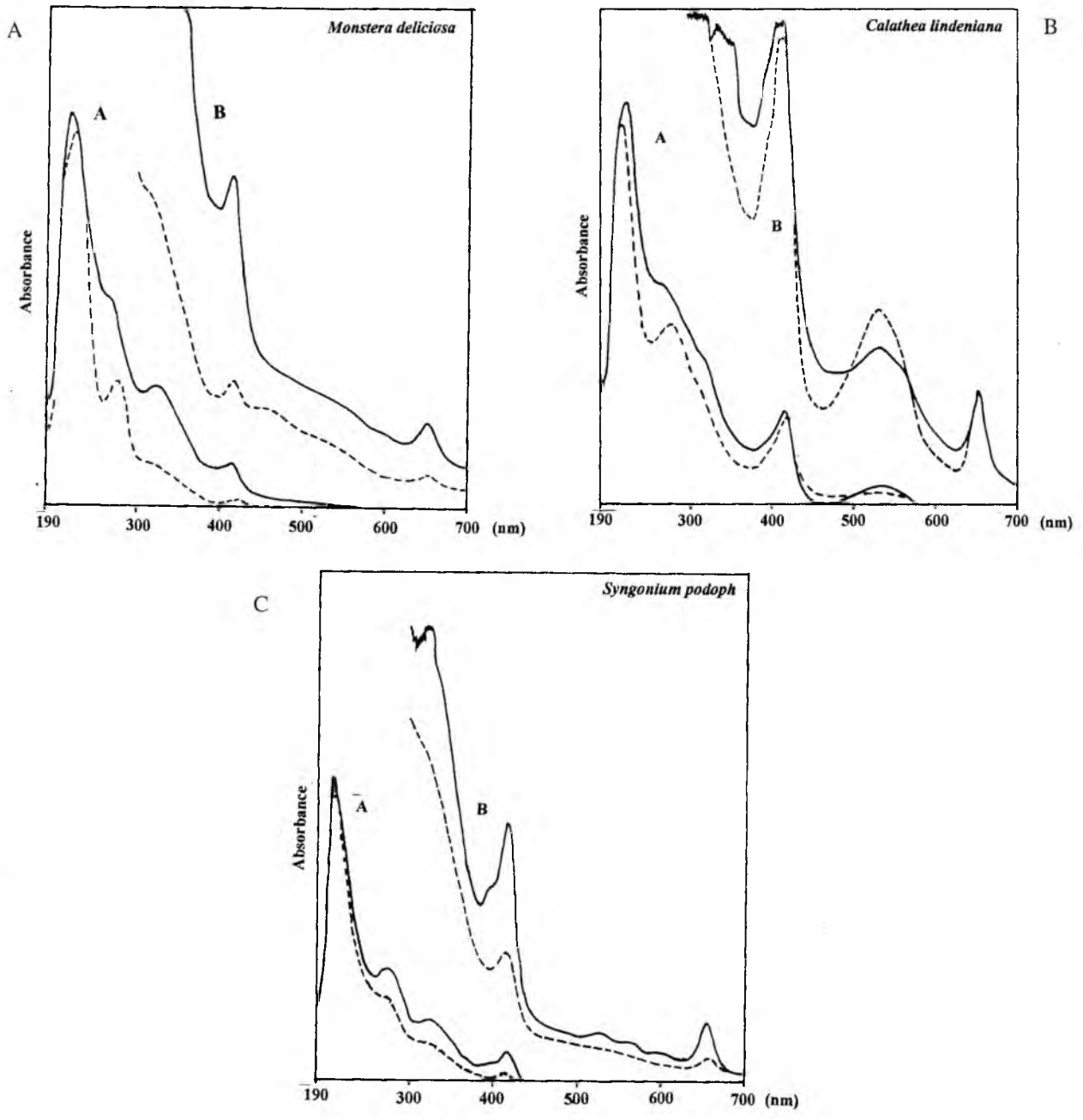

Figure 4. Effect of UV-B radiation (0.8 mW cm^{-2}) on flavonoids and phenolic compounds in control (-----) and treated (——) leaves of *Monstera deliciosa*, *Calathea lindeniana* and *Syngonium podoph*. Curve A, spectral scan taken with 1 : 9 dilution; Curve B, spectral scan taken with 1 : 4 diluted extract.

sclereids. Such structures can provide rigidness to the leaves, thereby preventing wilting due to osmotic loss under UV-B condition. Similar function may also be ascribed to the formation of sclerenchymatous tissue in the vascular system (Figure 2B).

The honey comb-like specialised structures which were seen in leaves of *S. podoph* were found more in case of UV-B-treated plant than in control. The structure could be fat or starch bodies which got accumulated under the UV-B exposure as an adaptive mechanism, however, it needs to be further studied to confirm the identity and function of these structures.

The absorption scan of phenolic extract from leaves were carried out using spectrophotometer. The UV-B treatment resulted in qualitative and quantitative changes in the phenolic compounds (Figure 4A–C). It was observed that absorption scan of UV-B-treated leaves of *M. deliciosa* showed five distinct peaks, out of which three were in UV region at 220 nm, 275 nm and 325 nm and two peaks were seen in visual region at 418 and 650 nm. Peaks at 220 nm (caumarin), 275 nm (flavonol) and 325 nm (flavone) in the UV region and 418 nm (anthocyanin), 650 nm (anthocyanin) in the visual region increased significantly in response to UV-B treatment (Figure 4A).

In *C. lindeniana*, more or less the same kind of changes in phenolic compounds were observed as seen in *M. deliciosa* (Figure 4B). The UV-B exposure led to the increase in phenolic compounds at 220 nm (caumarin), 275 nm (flavonol) and 325 nm (flavone) compared to control (Figure 4B). However, peaks in the visual region at 418 nm and 650 nm (anthocyanin) remained more or less the same compared to control. A slight decrease in the phenolic compound at 530 nm (anthocyanin) was observed in the UV-B-treated leaves (Figure 4B).

The UV-B treatment of *S. podoph* resulted in changes in phenolic compounds due to the UV-B treatment. Peaks at 215 nm (caumarin), 275 nm (flavonol) and 325 nm (flavone) in the UV region and peaks at 418 nm and 650 nm (anthocyanins) in the visual region all increased due to the treatment (Figure 4C). In addition, a slight peak at 565 nm and 585 nm (anthocyanins) was also observed as a result of the treatment (Figure 4C). Table 2 shows the comparative changes in the phenolic compound in all three plants studied.

The results indicate that flavonoids and other UV-B absorbing phenolic compounds are synthesized in response to UV-B treatment and get accumulated in the upper region of the leaf and provide protection against harmful UV-B radiation by absorbing the incoming radiation and thereby preventing the damage to inner-more sensitive tissues. Anthocyanin, in addition to act as a screening pigment (Tevini, 1999), may also play an important role as an antioxidant (Bjorman and Sundby, 1995) since UV-B radiation may also result in oxidative damage (Cen and Bjorn, 1994). Hahlbrock and Scheel (1989), Tevini *et al.* (1991), He *et al.* (1993) and Lois (1994) have reported that flavonoids and anthocyanin absorb UV-B radiation and these compounds are generally accumulated in the epidermis in order to prevent penetration of UV-B radiation to photosynthetic tissues and other sensitive sites. Wilson *et al.* (1998) have also reported accumulation of flavonoids in response to UV radiation in *Brassica napus*.

Figure 5 shows the effect of UV-B radiation on various plant pigments in *M. deliciosa*. A marked decline in all pigments was observed in UV-B-treated plants compared to control. Chlorophyll *a* and *b* showed much decrease in UV-B-treated plant. Xanthophylls such as lutein and violaxanthin were also

Table 2. Effect of supplementary UV-B radiation on comparative changes in phenolic compounds. '+' indicate presence and '–' indicate absence of the peaks. A slight shift seen in some of the λ_{max} is probably due to the glycosylation of phenolic compounds.

	λ_{max} (nm)											
	215–220		275		320–325		418		530		650	
Plant	C	T	C	T	C	T	C	T	C	T	C	T
M. deliciosa	+	++	+	+++	–	+++	+	++++	–	–	+	++
C. lindeniana	+	++	+	++	–	++	+	+	++	+	+	+
S. podoph	+	++	+	++	–	++	+	+++	–	+	+	++

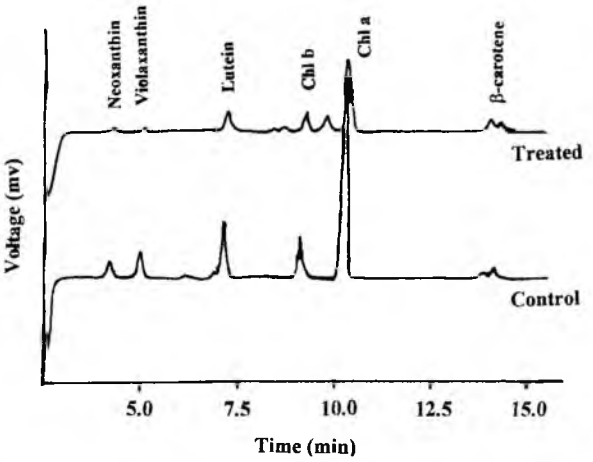

Figure 5. An HPLC profile of effect of UV-B radiation (0.8 mW cm^{-2}) on photosynthetic pigments in *Monstera deliciosa*.

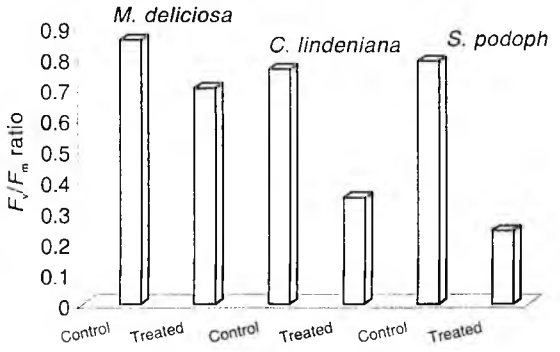

Figure 6. Effect of UV-B radiation (0.8 mW cm^{-2}) on F_v/F_m ratio in control and treated leaves of *Monstera deliciosa*, *Calathea lindeniana* and *Syngonium podoph*.

affected to a greater extent compared to control. Similar results were obtained with *C. lindeniana* and *S. podoph* (data not shown).

These results indicate that UV-B radiation may cause a damage to photosynthetic pigments. This damage could be of two types: one that UV-B radiation may directly cause photobleaching of pigments in the light-harvesting complex or it may damage the protein which house pigments (pigment–protein complexes). In our study we observed decrease in the F_v/F_m ratio in all three plants in response to UV-B treatment (Figure 6). The decrease in the F_v/F_m ratio was due to decrease in the F_o (indicative of decrease in the excitation energy reaching the photosynthetic reaction centre II due to loss of pigments in the light harvesting complex-II) as well as F_m (indicative damage to the PS II reaction centre itself). However, this aspect needs further study.

From this study it can be concluded that anatomical and biochemical responses reported in leaves of the three plants (*M. deliciosa, C. lindeniana* and *S. podoph*) studied here may occur in order to provide better protection against UV-B damage.

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