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Stress response of *Trichosanthes cucumerina* L. (Cucurbitaceae) to elevated UV-B doses

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UV-B radiation induced alterations in the contents of photosynthetic pigments, antioxidants (phenolics and ascorbic acid) and chlorophyll fluorescence in two tropical variants of *Trichosanthes cucumerina* L. – Cucurbitaceae. The Green (Variant A) and Light Green (Variant B) variants of *T. cucumerina*, which had earlier been shown to differ in their total phenolics contents, were used for the study. The plants were exposed to two UV-B doses of 11.9 kJ m^{-2} corresponding to 4 h, 23.8 kJ m^{-2} corresponding to 8 h and a control (without UV-B treatment). The maximum photochemical efficiency of the control plant (0.75) was significantly higher than those of the plants exposed to UV-B for 4 h (0.63) and 8 h (0.44). The chlorophyll *a* and total chlorophyll contents of the control plant were significantly higher than those of plants exposed to UV-B for 4 h and 8 h. The chlorophyll *b* contents were not significantly affected by the UV-B exposure. We report a significant decline in the total phenolics and ascorbic acid composition at 4 hours after exposure to UV-B. However, at 8 h after exposure, the total phenolics and ascorbic acid contents increased significantly compared to 4 h after exposure. The UV-B doses had no significant effect on the concentrations of Mg and micronutrients (Fe, Zn, Mn, and Cu) in the two variants of *T. cucumerina* used for this study. The physiological explanations for these results are discussed.

Key words: UV-B, stress, pigment, antioxidant, chlorophyll, fluorescence *Trichosanthes cucumerina*

Introduction

Ozone depletion raises concerns since the deleterious effects of UV-B radiation have been observed in various plant species. UV-B (280–315 nm) is an obligatory component of the solar spectrum, which is efficiently absorbed by plants, and also causes direct as well as reactive-oxygen-species -mediated damage to nucleic acids, proteins, lipids and plant pigments (BORNMAN et al., 1997, COCKELL and KNOWLAND 1999). Exposure of plants to elevated UV-B doses has been implicated in the induction of oxidative stress as a result of a process described as disturbance of the pro-oxidant-antioxidant balance in cells (SCHMITZ-EIBERGER and NOGA 2001). Some other studies have shown that a supra-optimal UV-B do-

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se could cause reduction in plant growth (SULLIVAN and TERRAMURA 1989), reduction of biomass production (TOSSERAMS et al., 2001), reduction of photosynthetic capacity (ZISKA et al., 1993; TERRAMURA and SULLIVAN 1994) and crop yield reduction (CORLETT et al. 1997). Reports by STRID and PORRA (1992), SULLIVAN and ROZEMA (1999) and BALAKRISHNAN et al. (2005) showed that elevated UV-B radiation caused reduction of photosynthetic pigment levels in plants. In *Phaseolus vulgaris* L., UV-B caused reduction in chlorophyll *a*, *b* and carotenoid levels (STRID and PORRA, 1992) while SCHMITZ-EIBERGER and NOGA (2001) recorded reduced chlorophyll *a*, *b* and total chlorophyll but enhanced malondialdehyde content over a 24 h period in UV-B treated bean. Numerous reports (CALDWELL, 1981, TEVINI et al. 1981, THOMA and IWANZIK 1983, DEMMIG-ADAMS and ADAMS 1992, LI et al. 1993, ROZEMA et al. 1997, MACKERNESS and THOMAS 1999, SCHMITZ--EIBERGER and NOGA 2001) showed that plants contain carotenoids, phenolics, flavonoids, flavones and alkaloids which function in the photo-protection of photosynthetic systems by dissipating excess excitation energy through the xanthophylls cycle.

According to the United States Environmental Protection Agency-USEPA (1995), the ozone layer is naturally thinner in the tropics than in the mid- and high-latitudes, so there is less ozone to absorb the UV radiation as it passes through the atmosphere. Under the assumption that solar UV-B radiation will reach peak levels on the Earth's surface in the next few years (KAKANI et al. 2003) and the reports of higher UV-B fluxes in the area around the equator (USEPA 1995), a study of this type is paramount to evaluate the degree of stress response and plant defense mechanisms under elevated UV-B in tropical crops such as *Trichosanthes cucumerina* L -Cucurbtaceae, a native plant of Nigeria. In this work we used two variants of *T. cucumerina* which had earlier been shown (ADEBOOYE 2007) to differ in their fruit pulp contents of antioxidants (carotenoids, phenolics and flavonoids). We therefore hypothesize that these two variants of *T. cucumerina* that are apparently adapted to the current UV-B level in tropical zone will show significant stress response to further increases in UV-B doses and that varietal differences in the responses will occur.

The UV-B induced by narrow band lamps (λ 311 nm) was calculated with Caldwell's generalized plant action spectrum (CALDWELL 1971) and resulted in values that give us realistic ideas about plant reactions to UV-B after ozone depletion. In this work, we report our findings on the differences in the leaf contents of pigments and antioxidants and the stress-response of leaves (evaluated by chlorophyll fluorescence) of *T. cucumerina* to elevated UV-B levels.

Materials and methods

Plant material

The study was carried out at the Institute of Crop Science and Resource Conservation-Horticultural Science- (INRES) University of Bonn, Germany. Stored seeds (99% viability) of two morphological variants of *T. cucumerina* produced in October 2006 were used for the study. The two morphotypes had earlier been described by ADEBOOYE et al. (2005): Variant A (Green) has long fruit with a deep green background and white stripes at unripe stage while Variant B (Light Green) has long, light green fruit when at unripe stage. The fruits of both morphotypes turn red at ripening.

Growth medium

The study was set up in a digitally controlled growth chamber (Model PVP, Phillips, Germany) maintained at 20 ± 1 °C air temperature, relative humidity of 60 ± 5% and supplied with sodium fluorescent lamps providing PAR of 160 μmol s^{-1} m^{-2} for a 14-hour-light period daily throughout the duration of the experiment. A 50 cm × 80 cm propagator (Model »Three-in-one-propagator«, Jemp Engineering Ltd., UK) equipped with adjustable heating control elements was used for raising the plants at 30 °C root zone temperature. The seeds were treated with Aatiram® fungicide (Stähler Agrochemie, Germany) at the rate of 0.5 g per 100 g seed before planting. Seeds were sown in plastic cups filled with peat-based compost which contained 4.0 mmol NO_3, 1.3 mmol P, 2.2 mmol K, 1.0 mmol Ca, 1.0 mmol Mg, 15.0 mmol Fe and 5.0 mmol Mn per liter. The propagator was stuffed with inert perlite to serve as insulator and conserve the heat so as to maintain the required root zone temperature. The plastic cups were inserted into the insulated propagator. There was one seedling per cup. Moisture level in the cups was maintained by applying water of the same temperature as the soil substrate itself every other day. The seedlings were monitored from emergence until the six-leaf stage of growth (four weeks=28 days after emergence) when they were subjected to UV-B exposure.

UV-B radiation treatment

UV-B stress was induced in an irradiation chamber with ten narrow-band (λ 311 nm) fluorescent lamps (Philips, TL 100 W/01) and ambient PAR intensity of about 100 μmol m^{-2} s^{-1}. The intensity of UV-B radiation was measured with a RM-21 spectroradiometer (Gröbel UV-Electronics, Ettlingen, Germany). In the experiment for assessment of dose--response relationships, plants were irradiated with UV-B fluxes of 22 W m^{-2} for 4 hours and 8 hours. These resulted in a total biological effective UV-B of 11.9 kJ m^{-2} and 23,8 kJ m^{-2} as weighted by Caldwell's generalized plant action spectrum (CALDWELL 1971).

Chlorophyll fluorescence (CF) measurement

Measurements of yield and maximum fluorescence were performed with a pulse amplitude modulation fluorometer (PAM, model 2000, Walz, Effeltrich, Germany). All measurements were done on the adaxial side of the largest fully expanded leaf after treatment of the leaves with UV-B and subsequent dark-adaptation of the leaves for 30 min. CF was measured on both plant species at a temperature of 20 °C as described earlier (FÖRSCHLER et al. 2003) and the ground state fluorescence yield, maximum fluorescence and relative photochemical efficiency were determined by the PAM 2000.

Pigment determination

Chlorophyll *a* and *b* and carotenoid contents of the leaves were extracted in dimethyl sulphoxide and determined spectrophotometrically (Perkin-Elmer Lambda 5/15) as described by SCHMITZ-EIBERGER and NOGA (2001)

Total phenol determination

Total phenol content was determined with the Folin-Ciocalteau method. In a 10 mL Eppendorf tube, 0.5 mL of distilled water, 500 μL of sample appropriately diluted and 500 μL of Folin Ciocalteau reagent were added and mixed. After 30 sec, 5 mL of aqueous sodium carbonate (20 %) were added and the mixture was incubated at room temperature in the dark for 30 min. The extinction was read at 720 nm, and the total phenol concentration was calculated from a calibration curve, using gallic acid as a standard. Results were expressed as mg/100 g fresh weight gallic acid equivalents.

Ascorbic Acid (AA) determination.

For determining the ascorbic acid content, leaves were homogenized with a dismembrator and extracted with potassium metaphosphate buffer. Two aliquots of each samples were measured, one sample containing ascorbic acid-peroxidase and the other without addition of the enzyme. Since ascorbic acid is an easily oxidizable compound, it was necessary to add the reducing agent 2,3-dihydroxybutane-1,4-dithiol during preparation. Analyses were performed according to a standardized procedure (SCHMITZ-EIBERGER and NOGA 2001).

Cations determination.

After drying the plant material in a lyophilizer, samples were ground to a fine powder, and 0.3 g of the dried sample were digested with HNO_3 and H_2O_2 according to CHEN et al. (1997). Analyses of Fe, Mn, Zn and Cu were done by atomic absorption spectrophotometry.

Data Analysis

All data collected were subjected to analyses of variance using the standard method for factorial experiment by using the SPSS package (Superior Performance Software System version 14.0.). Two independent experiments were conducted. For each experiment, triplicate determination of each parameter was carried out on the Variants A and B independently. Means, where significant were separated using the least significant difference at 5% level of probability.

Results

Chlorophyll contents and chlorophyll fluorescence as affected by UV-B doses

The hypothesis that tropical *T. cucumerina* L. would show significant stress response to elevated UV-B doses was validated by the findings in this study. The UV-B doses induce significant stress responses as documented by the evaluation of chlorophyll fluorescence and the contents of chlorophyll *a*, total chlorophyll, ascorbic acid and total phenolics while the chlorophyll *b* content was not significantly affected (Fig. 1 a-g). The control plants (without UV-B) had significantly lower Fo (ground state fluorescence) compared to plants in 4 hours UV-B exposure while plants at 8 hours UV-B exposure showed higher Fo level than those at 4 hours UV-B exposure (Fig. 1a). The maximum fluorescence yield of the

plants exposed to 8 hours UV-B treatment was significantly lower than of those exposed to 4 hours UV-B treatment while plants under control treatment recorded the highest maximum fluorescence yield value (Fig. 1b). Also, as measured by Fm, Variant A was more stressed than Variant B at 8 h after UVB exposure. The relative photochemical efficiency of the control plant (0.75) was significantly higher than those of the plants exposed to UV-B for 4 h (0.63) and 8 h (0.44) (Fig. 1c). The relative photochemical efficiency values for the two variants at each exposure level did not differ significantly. The chlorophyll *a* content of the control plant (without UV-B) was significantly higher (~28%) than that of the plants exposed to UV-B for 4 h and 8 h (Fig. 1d). The same trend was observed for total chlorophyll (Fig. 1e). The chlorophyll *b* content was however not significantly affected by the UV-B exposure but the values are higher under control treatment than in UV-B treated plants (Fig. 1f).

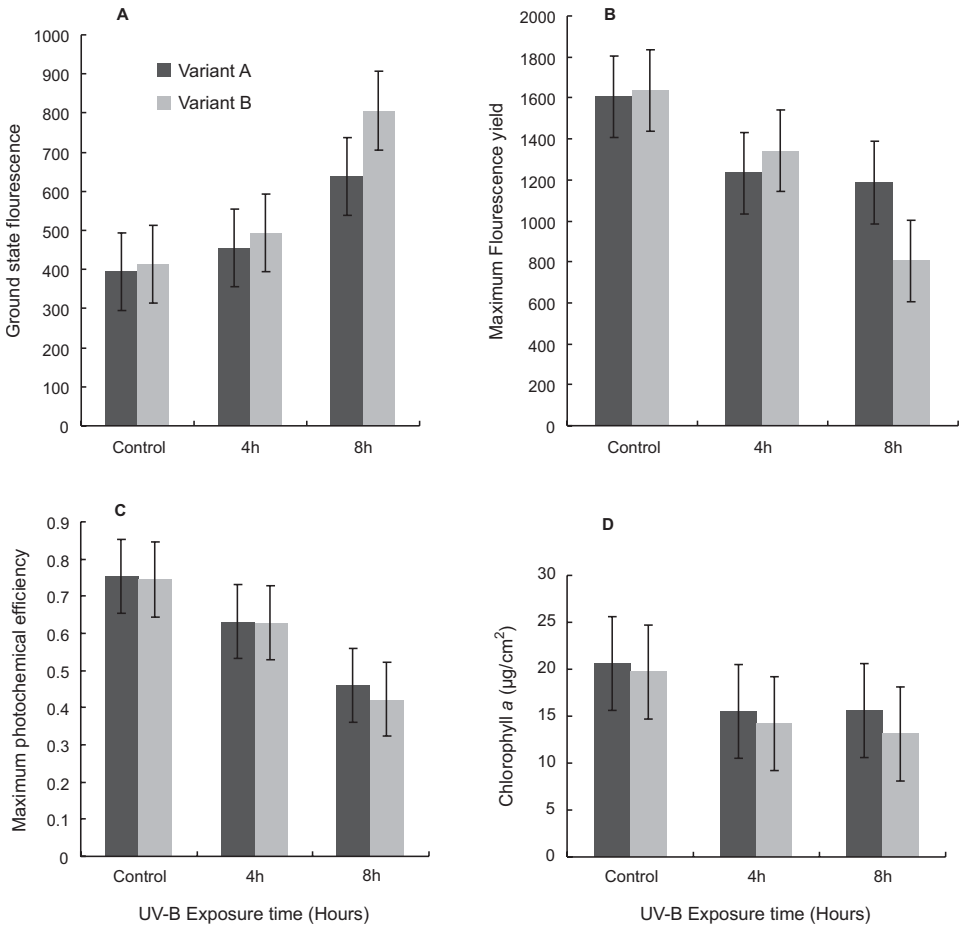

Fig. 1. a-d. Interactive effect of UV-B exposure time and *T. cucumerina* variants on ground state fluorescence (a), maximum fluorescence yield (b), maximum photochemical efficiency (c), chlorophyll *a* (d).

Total phenolics contents in the UV-B affected variants

The total phenolics content of the leaf of Variant A was significantly higher than that of Variant B under the control treatment (Fig. 1g) and this is in concordance with earlier findings by Adebooye (2007). Also, the results obtained clearly confirm our hypothesis that varietal differences in the responses to UV-B radiation will occur. There was a significant sharp decline (~48%) in the total phenolics of the two variants of *T. cucumerina* at 4 h after exposure to UV-B (Fig. 1g). However, at 8 h after exposure, the total phenolics contents increased (~22%) compared to samples taken at 4 h after exposure. However, in an attempt to combat the stress-effect and recover, there was apparently stimulation in phenolics synthesis which resulted in higher phenolics content at 8 h after exposure compared to 4 h after exposure. It is noteworthy that despite the increase in phenolics contents after 8 hours of

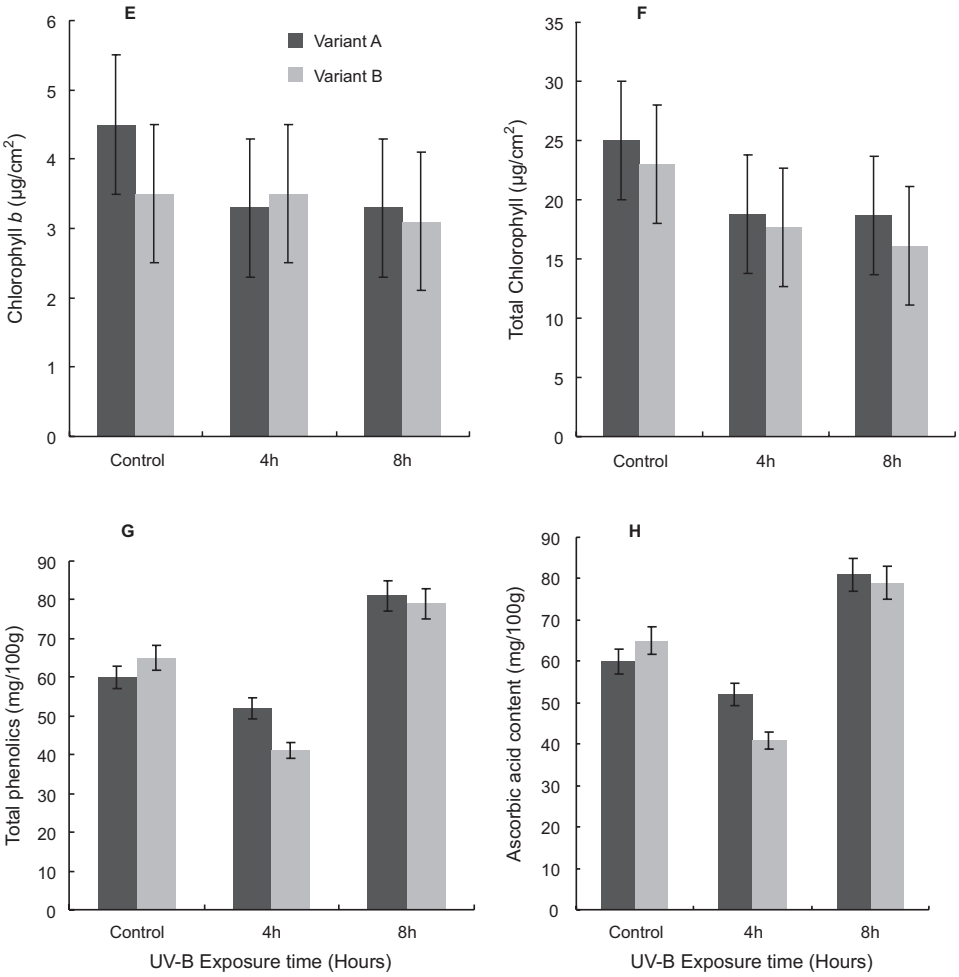

Fig. 1. e-h. Interactive effect of UV-B exposure time and *T. cucumerina* variants on chlorophyll *b* (e), Total chlorophyll (f), Total phenolics (g) and ascorbic acid contents of leaves (h).

UV-B treatment, the value was still lower than that of the control plants. It was also noted that the phenolics contents of the two variants were almost the same at 4 h after exposure while at 8 h after exposure the value was higher in Variant A. A clear inference from this study is that Variant A, which had higher total phenolics content, also showed more UV-B stress tolerance, as measured by maximum fluorescence yield and relative photochemical efficiency, at 8 h after UV-B exposure.

Ascorbic acid contents as affected by UV-B doses

As observed for the phenolics composition, the ascorbic acid composition also declined at 4 hours after exposure to UV-B radiation while at 8 hours after UV-B exposure the ascorbic acid levels increased again, even above the quantities recorded in the control plants (Fig. 1h). The initial decline in ascorbic acid contents could be explained as a major response to sudden stress. However, as a defense mechanism to counteract the stress effect, the plant responded by stimulating the synthesis of antioxidant (ascorbic acid).

Mineral nutrients composition as affected by variant and UV-B doses

We observed that the two variants of *T. cucumerina* did not differ significantly in their Fe, Zn, Mn and Cu contents with average values of 3.5, 1.1, 3.2, 1.1, and 0.3 mg per 100g fresh weight, respectively. However, the Mg content of Variant A (197.1 mg/100g) was significantly higher than that of Variant B (133.9 mg/100g). Generally, the UV-B exposure did not significantly affect the Mg and micronutrients (Fe, Zn, Mn and Cu) concentrations of the two variants of *T. cucumerina.* We suspected that this result was due to the short period of exposure of the plants to elevated UV B. We postulate that under longer term exposure to UV-B, the nutrient absorption mechanism may be affected. In a follow-up study therefore, we intend to elucidate the mechanism of mineral nutrient uptake under elevated UV-B exposure and also undertake investigations on mineral nutrition in relation to prolonged UV-B exposure.

Discussion

An increase in Fo was earlier interpreted as a reduction of the 'rate-constant' of energy trapping by PS II centres (HAVAUX 1993), which could be the result of a physical dissociation of light harvesting complex from PS II core (ARMOND et al. 1980). The decline in the Fv/Fm value as the time of exposure increased is an indication that certain damage had occurred in the photosystem II (PS II) reaction centres as a result of UV-B stress. In addition to the general damage to PS II, also D1 and D2 protein subunits of PS II reaction centres may degrade due to UV-B radiation (MELIS et al. 1992, FRISO et al. 1994). It has been reported that in healthy leaves, the maximum photochemical efficiency is close to 0.8 (BJÖRKMAN and DEMMIG 1987, FRACHEBOUD et al. 1999). A lower value is an indication that a proportion of PS II reaction centres may have been damaged, a phenomenon called photochemical-inhibition, often observed in plants under stress (LAASCH 1987). An enhanced UV-B radiation was shown to have negative impacts on photosynthetic activity (ZHAO et al. 2004).

An earlier study by SCHMITZ-EIBERGER and NOGA (2001) on *Phaseolus vulgaris* showed significant reduction in chlorophyll *a* and chlorophyll *b* following elevated UV-B exposure

for 2 h. In a situation of oxidative stress imposed by UV-B radiation, CAMPBELL (1975) reported that the chloroplast is the first organelle to show injury response, hence there is always a sharp reduction in the chlorophyll contents of leaves of UV-B treated plants. Several mechanisms have been suggested for the decline in chlorophyll contents of UV-B treated plants. TERRAMURA (1983) suggested that chlorophyll reduction in UV-B treated plants may be related to inhibition of biosynthesis or due to degradation of chlorophylls and their precursors, while STRID et al. (1994) linked reduced chlorophylls to reduced synthesis resulting from reduced expression of genes encoding chlorophyll-binding proteins. In another study, STRID and PORA (1992) suggested that accumulation of chlorophyllide *a* and *b* resulted in decreased chlorophylls contents under UV-B stress.

Most studies have reported significant increases in phenolics compounds and their derivatives in UV-B treated plant compared to control plants (DEMMIG-ADAMS and ADAMS 1992, LI et al. 1993, ROZEMA et al. 1997, AH-MACKERNESS and THOMAS 1999, SCHMITZ-EIBERGER and NOGA 2001, MUSIL et al. 2002, YAGINUMA et al. 2003, BALAKRISHNAN et al. 2005). Syntheses of phenolics compounds and their derivatives are activated under UV-B exposure to serve as defence mechanism and internal screens against UV-B damage (TEVINI et al. 1991, DAY 1993, HOQUE AND REMUS 1999). The crude optical properties of the phenolics with high radiation absorbance in the UV region and an excellent transmittance in the photosythetically active region of the spectrum (STEPHANOU and MANETAS 1997), combined with the mainly superficial location on the cuticle (WOLLENWEBER and DIETZ 1981), trichomes (KARABOURNIOTIS et al. 1992) or epidermis (ROBBERECHT and CALDWELL 1978), make them useful in plant defense against UV radiation (CALDWELL et al 1983).

We reported significant increases in ascorbic acid composition following UV-B exposure. SMIRNOFF (1996) in his review of ascorbic acid metabolism reported that cell wall ascorbate provides a first line of defense against ozone and sulphur dioxide. Ozone fumigation has been shown to increase ascorbate and ascorbate-glutathione cycle enzyme activity (CASTILLO and GREPPIN 1988). The biochemical functions of ascorbic acid in plants have been categorized into four: antioxidative role (harmful radical scavenger), enzyme cofactor, electron transport and oxalate/tartrate synthesis (SMIRNOFF 1996). Some studies have also suggested that the protective effect of ascorbic acid in stressed plants is more related to the reduction in the level of damage caused by active-oxygen species to essential proteins and/or nucleic acids (INZÉ and VAN MONTAGUE 1995, BECANA et al. 1998, NOCTOR and FOYER 1998). KERK et al. (2000) related mitotic quiescence in root meristems to low ascorbic acid levels.

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