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Effect of UV-B radiation on antioxidant enzymes and its modulation by benzoquinone and α-tocopherol in cucumber cotyledons

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The activity of antioxidant enzymes superoxide dismutase, ascorbic acid peroxidase, guaiacol peroxidase and glutathione reductase are enhanced in cucumber cotyledons in response to UV-B radiation. External application of antioxidants like α-tocopherol and benzoquinone suppressed the UV-B induced response of all the enzymes except guaiacol peroxidase. Free radicals generated by UV-B are likely to be involved in the induction of antioxidant defence system. External application of antioxidants altered the UV-B-induced enzyme activity, which might be due to their ability to scavenge free radicals.

EXPOSURE of plant tissues to UV-B radiation generates reactive oxygen species (ROS) and enhances the level of malondialdehyde formation in cucumber cotyledons[1,2] and in *Arabidopsis thaliana*[3] exposed to UV-B radiation. Plant cells are known to have both enzymatic and non-enzymatic defence mechanisms to counteract the destructive effects of activated oxygen species. The antioxidant defence system consists of low molecular weight antioxidants such as ascorbate, glutathione, α-tocopherol and β-carotenoids[4] as well as several antioxidant enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (POD), ascorbic acid peroxidase (APX) and glutathione reductase (GR)[5,6]. In the present work the effect of UV-B on the antioxidant enzymes and its modulation by α-tocopherol and benzoquinone have been examined.

Seeds of cucumber (*Cucumis sativus* L. var. long green) were obtained from Suttons and Sons Ltd, Kolkata. Seeds of uniform size and shape were selected, rinsed with 0.01% $HgCl_2$, washed thoroughly under tap water and finally with distilled water. Seeds were spread on moist filter paper on 15 cm petri dishes and grown in complete darkness at 25 ± 1°C for 48 h. For external application of antioxidants, seedlings were transferred in benzoquinone (0.01–1.00 mM) or α-tocopherol (25–300 μM). Benzoquinone and α-tocopherol were dissolved in ethanol and the solutions were applied to two layers of Whatman filter paper no. 1 in 15 cm petri dishes. After evaporation of the solvent, the filter paper in petri dishes were wetted with 10 ml of distilled water and the seedlings were incubated in darkness for 24 h.

Cotyledons were excised from these seedlings grown for 24 h in darkness in different concentrations of benzoquinone and α-tocopherol. Six cotyledons were floated with their inner surface exposed in each of the 9 cm petri dishes containing 10 ml of distilled water and grown in complete darkness or exposed to UV-B (2.6 mW/cm^2) at 25 ± 1°C. The antioxidant enzymes, except guaiacol peroxidase, were assayed immediately after the termination of UV-B exposure. Guaiacol peroxidase activity was estimated either immediately or after 24 h of incubation in darkness after termination of exposure.

Transfer of seedlings and excision of cotyledons in the dark room (25 ± 1°C) were performed under a green safe lamp (Phillips 25 W covered with eight layers of green cellophane; irradiation at the level of seedlings being 0.2 W/m^2). Each experiment was run with triplicate sets of cotyledons; the values presented with standard errors are the mean of three experiments.

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UV-B (280–320 nm) was obtained from FL-20-SE, Toshiba, Tokyo (λ-max = 311 nm; 40 W). UV-B was filtered through a polyvinyl chloride film (UV-C-O Mitsuioatsu Ltd, Japan). Irradiance at the level of cotyledons was 2.6 mW/cm^2, measured with UV-Tex a + b idm radiometer [Optix Tex. Inc., Washington D.C.].

SOD activity was assayed according to the method of Beauchamp and Fridovich[7]. The reaction mixture contained 0.24 mM riboflavin, 2.1 mM methionine, 1% Triton × 100, 1.72 mM nitroblue tetrazolium chloride (NBT; in 50 mM sodium phosphate buffer, pH 7.8) and 200 µl of enzyme extract (in 50 mM Tris HCl buffer, pH 7.8). SOD activity was expressed as units/mg protein. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition in the rate of NBT photo-reduction.

APX activity was measured by the method of Nakano and Asada[8]. The reaction mixture contained 2.5 ml sodium phosphate buffer (pH 7.4, 50 mM), 0.3 mM ascorbate, 0.06 mM EDTA, 300 µl enzyme extract (in 50 mM sodium phosphate buffer, pH 7.4) and 200 µl of 2 mM H_2O_2 in a total volume of 3 ml. The decrease in absorbance at 290 nm (extinction coefficient 2.8 mM^{-1} cm^{-1}) was recorded at 25°C for 1 min. The activity was calculated as mmol ascorbic acid (AA) oxidized/min/mg protein.

GR activity was determined by the method of Rao *et al.*[9]. The reaction mixture contained potassium phosphate buffer (50 mM, pH 7.8), 10 µl NADPH (1 mM), 100 µl oxidized glutathione (15 mM) and 0.3 ml enzyme extract (potassium phosphate buffer 100 mM, pH 7.8). GR activity was expressed as µmol NADPH oxidized/min/mg protein following decrease of absorbance at 340 nm.

POD was assayed by the method of Maehly[10]. The reaction mixture contained 0.5 ml enzyme extract (in 0.02 M, phosphate buffer, pH 6.4), 1 ml of 20 mM guaiacol, 3 ml of 0.02 M phosphate buffer and 0.03 ml H_2O_2 (88.2 mM). The activity was determined as the change in OD at 475 nm/min/mg protein. Protein was estimated by the method of Lowry *et al.*[11] using BSA as the standard.

In response to UV-B the activities of the antioxidant enzymes SOD, APX, GR and POD were enhanced in the cotyledons of cucumber. Pretreatment of cotyledons with tocopherol or benzoquinone suppressed the UV-B-induced enhancement of SOD activity in the cotyledons (Figure 1 *a* and *b*). UV-B (2.6 mW/cm^2, 1 h) enhanced the activity of SOD by about ca. 200%, which was suppressed by benzoquinone in a concentration-dependent manner (0.001 to 1.0 mM). At lower concentrations of benzoquinone, there was a slight promotion of the SOD activity even in the untreated controls (Figure 1 *b*). In contrast to this, α-tocopherol suppressed the activity both in the control and UV-B-treated cotyledons, although a clear concentration dependence was not discernible (Figure 1 *a*). Both benzoquinone and α-tocopherol suppressed the activity of ascorbic acid peroxidase (Figure 2 *a* and *b*). A concentration response was more evident in the case of benzoquinone. UV-B-enhanced GR activity was similarly suppressed by the antioxidants (Figure 3 *a* and *b*). The effect of antioxidants on POD activity was quite different compared to

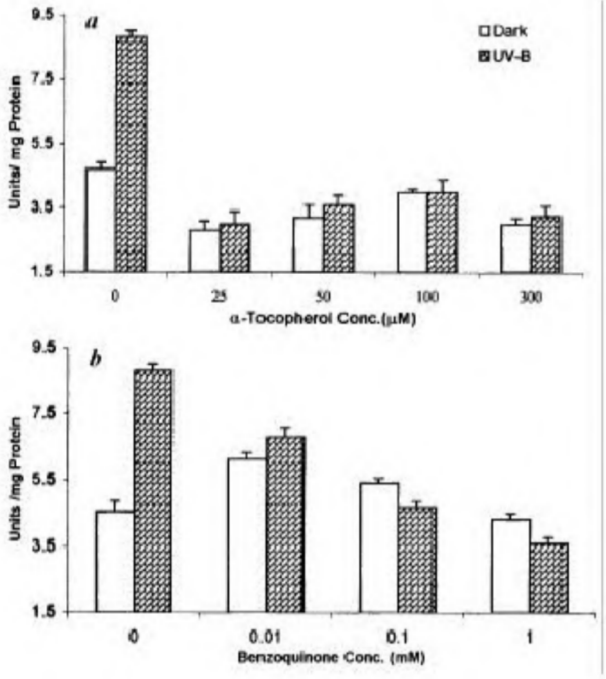

Figure 1. Effect of different concentrations of α-tocopherol (***a***) and benzoquinone (***b***) on SOD activity in dark or UV-B-exposed (1 h, 2.6 mW/cm^2) cucumber cotyledons at 24 h. Vertical bars indicate SE for mean.

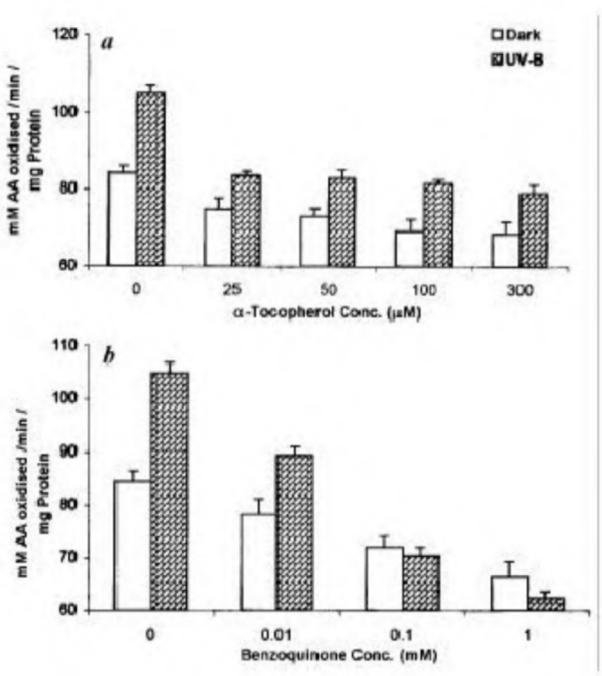

Figure 2. Effect of different concentrations of α-tocopherol (***a***) and benzoquinone (***b***) on APX activity in dark or UV-B-exposed (30 min, 2.6 mW/cm^2) cucumber cotyledons at 24 h. Vertical bars indicate SE for mean.

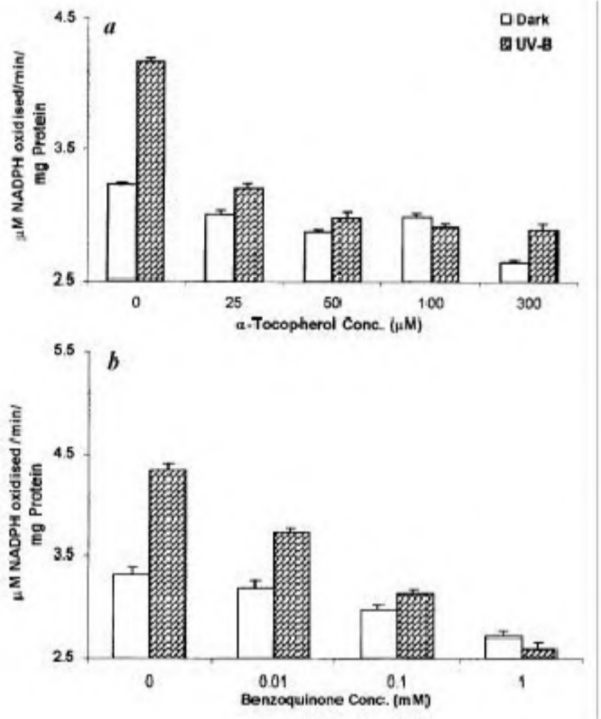

Figure 3. Effect of different concentrations of α-tocopherol (*a*) and benzoquinone (*b*) on GR activity in dark or UV-B-exposed (1 h, 2.6 mW/ cm^2) cucumber cotyledons at 24 h. Vertical bars indicate SE for mean.

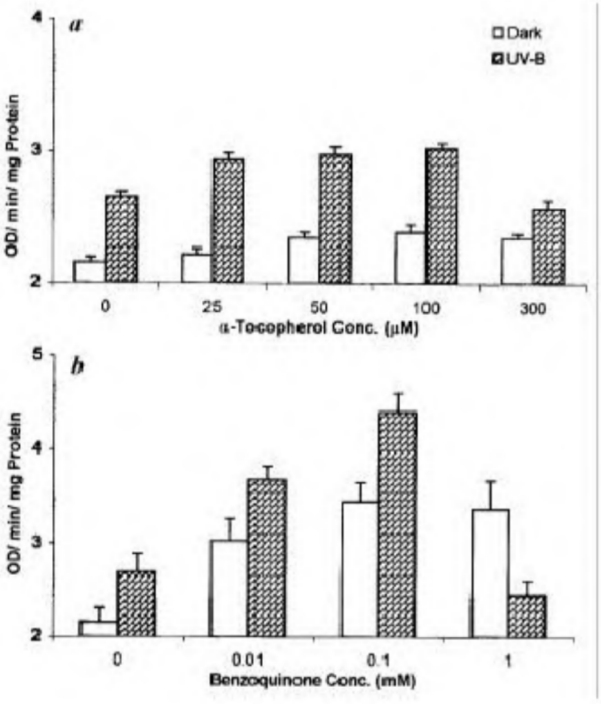

Figure 4. Effect of different concentrations of α-tocopherol (*a*) and benzoquinone (*b*) on POD activity in dark or UV-B-exposed (1 h, 2.6 mW/ cm^2) cucumber cotyledons at 24 h. Activity was assayed immediately after termination of UV-B exposure. Vertical bars indicate SE for mean.

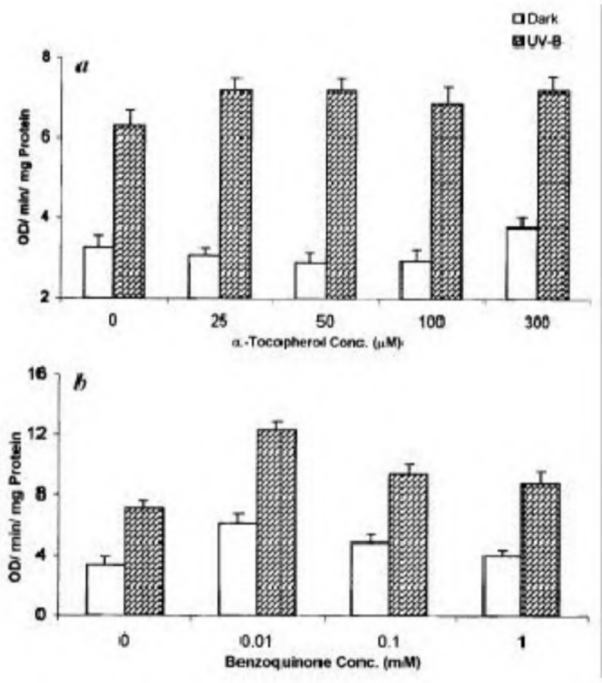

Figure 5. Effect of different concentrations of α-tocopherol (*a*) and benzoquinone (*b*) on POD activity in dark or UV-B-exposed (1 h, 2.6 mW/ cm^2) cucumber cotyledons at 24 h. Activity was assayed at 24 h after termination of UV-B exposure. Vertical bars indicate SE for mean.

other antioxidant enzymes. POD activity was measured at two stages, either immediately or after 24 h of incubation in the dark after the termination of UV-B exposure. Both the antioxidants enhanced the level of UV-B-induced POD activity prominently immediately after the termination of UV-B exposure (Figure 4 *a* and *b*). The enhancement due to application of antioxidants was less prominent at 24 h after the termination of UV-B exposure (Figure 5 *a* and *b*).

Plant tissues maintain a delicate balance between rates of free-radical generation and their removal by evolving elaborate regulatory mechanisms to control the synthesis of antioxidant enzymes in response to different oxidative stimuli. Activation of antioxidant enzymes by UV-B has earlier been observed in *A. thaliana*[9], wheat[12] and cucumber[13]. Activation of antioxidant enzymes by UV-B in the presence of externally supplied antioxidants was not observed in earlier studies.

In the present study, α-tocopherol and benzoquinone lowered the UV-B-enhanced activity of SOD, APX and GR. An indication about the involvement of oxyradicals in enhancing the activity of antioxidant enzymes emerges from the experimental data presented here. When the cucumber seedlings are grown in benzoquinone or α-tocopherol before UV-B exposure, enhancement in the antioxidant enzymes is not observed on subsequent exposure to UV-B. Thomsen *et al.*[14] have observed that pretreatment of mustard seedlings with benzoquinone and α-tocopherol suppresses APX activity induced by red light under photoxidative stress.

Although the antioxidants suppressed the activity of SOD, APX and GR, they had a promoting effect on peroxidase. Both benzoquinone and α-tocopherol enhanced peroxidase activity in the cotyledons exposed or unexposed to UV-B. This observation needs further experimentation.

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Status, utility, threats and conservation options for rattan resources in Manipur

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Manipur, one of the eight states of northeastern India, harbours more than 13 species of rattans under three genera as against 51 species under five genera from the rest of India and 600 species under 13 genera from the world as a whole. Rattans are mainly used for construction, craft, fencing, rituals, rope, food, medicine, etc. In Manipur, about 15×10^4 and 293×10^4 running metres of canes were extracted officially during 2000–01 and 1992–93 respectively. The revenue generated from rattans and bamboos was 6.2% (out of which 0.6% was from rattans) of total minor forest products during 1999–2000. During 2000–01, a revenue of Rs 13,000 was generated from rattans against Rs 740,000 during 1992–93. The extraction pressure was 6.8 running metre of cane per km^2 during 2000–01 as against 131 running metre of cane per km^2 during 1992–93. Continuous and unscientific extraction of rattans from the vast natural habitat, threatens their survival in Manipur. Cultivation of rattans in Manipur and by and large in the whole of northeastern India, is a challenge that needs to be attended to urgently.

RATTANS, prickly climbing palms or canes with solid stem, form a group of 600 species distributed throughout Southeast Asia and the neighbouring areas and are principal non-timber forest products[1]. They are highly valued and have social and economic importance because of their unique characteristics such as strength, durability, looks and bending ability; they are regarded as 'green gold'[1]. In 1996, about 80% of the rattans at the international market originated from Indonesia and in 1999, the export volume was 590,021 tons and a value of US $ 1.147 billion[2]. Cane and bamboo craft is traditional and symbolic of northeastern India. The role of bamboo in the socio-economy of the people of Manipur has been studied[3]. Rattans are produced in the evergreen tropical and moist deciduous forests of northeastern India, Kerala, Karnataka and Tamil Nadu[1] for the manufacture of furniture and other articles in India.

Nestled in a valley among the hills of eastern Himalayas, far above the sea level with nature's pristine glory, Manipur's mythological concept of creation is revealed in her famous folk dances and crafts[4]. Cane handicraft has an

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