Protective Mechanism in UV-B treated *Crotalaria juncea* L. Seedlings

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Abstract

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There is concern that some anthropogenic atmospheric pollutants may result in a global reduction of stratospheric ozone. This would undoubtedly increase the level of ultraviolet radiation reaching the surface of the earth, which might have important biological consequences. The antioxidant defence system of a plant consists of a variety of antioxidant molecules and enzymes. The role of antioxidant enzyme activities in *Crotalaria juncea* under control without UV-B treatment and ultraviolet-B supplemental radiation (UV-B) was investigated. UV-B treatment for 6 h for 4 days resulted in severe inhibition in catalase activity. On the other hand, the activities of peroxidase, polyphenol oxidase, superoxide dismutase and phenylalanine ammonialyase increased after the UV-B treatment when compared to control seedlings. These increases could be an adaptive mechanism to minimise the effects of UV-B radiation.

Keywords*: Crotalaria juncea* L.*;* UV-B radiation; antioxidant; catalase

Abbreviations: UV-B – Ultraviolet-B; O_2^-,– Oxyradical; PVP – Polyvinyl pyrolidone

Recent research has shown that global stratospheric ozone, the primary attenuator of solar ultraviolet radiation, has significantly decreased during the last decade. Manmade chemicals such as chlorofluorocarbons, invented in the 1930s, are the main culprits for the depletion of the stratospheric ozone layer (Frederick 1990). A depletion by about 20% of stratospheric ozone corresponds to a 20% increase in the flux of biologically damaging UV-B radiation (280–320 nm).

There is an urgent need of reevaluating the UV-B radiation effects on organisms under realistic irradiation protocols. This has been the aim of extensive research in recent years, especially on plants (Ravindran *et al*. 2001; Brenda 2002). Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. They are continuously produced by an organism during normal use of oxygen such as respiration and some cell-mediated immune functions. These free radicals are also generated through environmental stress (Tiwari 2004).

As a result of ozone loss, UV-B flux at the surface of the earth inevitably increases the negative impacts on organisms (Coohill 1991). Exposure to high UV-B radiation alters photosynthetic enzyme activities (Nedunchezhian & Kulandaivelu 1991), disrupts PS2 reaction centres (Iwanzik *et al*. 1983) and modifies stomatal closure (Negash 1987). Growth characteristics are also altered in plants that are UV-B sensitive. UV-B radiation supplied either artificially or naturally has resulted in

decreased stem length, leaf area and plant height in cucumber, sunflower, soybean and loblolly pine (Tevini & Teramura 1989). Reduction in biomass accumulation resulting from increased UV-B radiation was observed in wheat, barley, soybean, tomato, cucumber and lettuce (Krupa & Kickert 1989). However, defence mechanisms such as foliar symptoms like curling of leaves and shiny wax coating (Nedunchezhian & Kulandaivelu 1996), synthesis of phenolic substances such as anthocyanin and flavonoids (Ravindran *et al*. 2001) and synthesis of antioxidant enzymes like peroxidase, polyphenol oxidase and superoxide dismutase (Rao *et al*. 1996) have been observed in UV-B treated *Arabidopsis thaliana* seedlings. Many plants possess very efficient scavenging systems for reactive oxygen that protects them from destructive oxidative reactions (Arora *et al*. 2002).

UV-B radiation increases the production of reactive oxygen species (H_2O_2, O_2^-, OH^-). These oxygen species are extremely reactive and have a cytotoxic nature (Bowler *et al*. 1992). Plants have evolved protective mechanisms to keep these deleterious reactions to a minimum. Antioxidative enzymatic defence includes catalase, peroxidase and superoxide dismutase. Polyphenol oxidase and phenylalanine ammonialyase also participate in protection via phenolic compounds. These enzymes can mitigate the UV-induced damage by protecting the photosynthetic pathway and cellular components. The present study was conducted to determine the role of antioxidant defence mechanism in UV-B treated seedlings of *Crotalaria juncea* L., a plant widely cultivated in South India as green manure.

MATERIALS AND METHODS

Plants. Pre-soaked seeds of *Crotalaria juncea* were germinated in the dark for 2 days and then transferred to indirect daylight in the laboratory. After 2 days, when the primary leaves had fully expanded, the seedlings were given appropriate treatment in an irradiation chamber at 28°C (Hot Pack Crop, U.S.A.).

Radiation treatment. Seedlings were exposed to radiation (12.2 KJ/m^2/day) for 6 h (10:00–16:00 h) for 4 day from four Philips 20W fluorescent tubes (type: TL/33) plus one Philips 20W/12 sunlamp (UV-B treated), or from four 20W white fluorescent tubes (control). Radiation below 280 nm was completely removed by using cellulose diacetate filter.

Samples of irradiated and control leaves were analysed for enzyme activity daily.

Extraction method for catalase. Catalase activity was assayed by measuring the rate of disappearance of H_2O_2 following the procedure of Maehly and Chance (1959). One gram of leaf sample was homogenised in 10 ml of 0.1M sodium phosphate buffer pH 7 and centrifuged at 4°C for 10 min at 10 000 g. An aliquot of one ml of the supernatant of the enzyme extract was added to the reaction mixture containing one ml of 0.01M H_2O_2, 3 ml of 0.1M sodium phosphate buffer having pH 6.8. The reaction was stopped after an incubation of 5 min at 20°C by addition of 10 ml of 1% H_2SO_4. The acidified medium without or with the enzyme extract was titrated against 0.005N $KMNO_4$.

Extraction method for peroxidase, polyphenol oxidase and superoxide dismutase. Peroxidase and polyphenol oxidase activities were determined by methods described by Kumar and Khan (1982). Superoxide dismutase activity was determined by the following method of Beauchamp and Fridovich (1971). One gram of leaves was homogenised with 20 ml of ice-cold extraction medium containing 2mM $MgCl_2$, 1mM EDTA. 10mM β-mercaptoethanol, 7 % PVP and 10mM sodium metabisulphate. The homogenate was strained through two layers of cheese cloth and centrifuged at 10 000 g for 15 min and the supernatant was made up to 20 ml with the same buffer and was used as the source of enzyme.

Extraction method for phenylalanine ammonialyase. Phenylalanine ammonialyase was extracted according to the technique proposed by Amrhein and Zenk (1971). Phenylalanine ammonialyase preparations were obtained by homogenisation of *Crotalaria* leaves in fluid nitrogen and extracted with buffer.

Statistics. The data were analysed by using analysis of variance (ANOVA). Multiple comparisons between treatment and control were done with the help of Tuckey's test (1953).

RESULTS

Crotalaria juncea seedlings grown under control or UV-B radiation showed much different enzymatic activities (Table 1). Catalase activity was reduced in UV-B treated seedlings by 30.3% after the 4 days treatment. In contrast, peroxidase activity was increased by supplemental UV-B radiation. The increasing trend reached a maximum

Table 1. Effect of supplemental UV-B radiation on catalase, peroxidase, polyphenol oxidase, superoxide dismutase and phenylalanine ammonialyase in *Crotalaria juncea* L. seedlings

Time (days)	Treatment	Catalase (μmol H_2O_2 decomposed min/g fr. wt.)	Peroxidase (μmol purpurogallin formed min/g fr. wt.)	Polyphenol oxidase (μmol purpurogallin formed min/g fr. wt.)	Superoxide dismutase (units h/mg protein fr. wt.)	Phenylalanine ammonialyase (μmol annamic acid formed g/h fr. wt.)
1	Control	11.45 ± 0.013	34.59 ± 0.174	28.09 ± 0.152	15.52 ± 0.019	12.27 ± 0.014
	UV-B	10.31 ± 0.017 (–9.90)	39.79 ± 0.177 (15.03)	30.95 ± 0.157 (10.18)	17.57 ± 0.023 (13.20)	13.75 ± 0.016 (12.06)
2	Control	14.02 ± 0.013	36.01 ± 0.175	30.14 ± 0.156	18.37 ± 0.022	15.31 ± 0.017
	UV-B	12.34 ± 0.019 (–11.98)	48.38* ± 0.186 (34.35)	37.50* ± 0.162 (24.42)	22.35* ± 0.029 (21.67)	18.02* ± 0.022 (17.70)
3	Control	16.08* ± 0.014	39.09 ± 0.179	32.26 ± 0.159	22.41* ± 0.027	18.42* ± 0.021
	UV-B	12.48 ± 0.020 (–22.39)	56.82* ± 0.192 (45.36)	42.95* ± 0.168 (33.14)	30.85* ± 0.038 (37.66)	23.02* ± 0.027 (24.97)
4	Control	18.30* ± 0.016	42.01 ± 0.182	34.50* ± 0.164	25.18* ± 0.034	22.01* ± 0.026
	UV-B	12.76 ± 0.022 (–30.27)	69.09* ± 0.197 (64.46)	52.45* ± 0.177 (52.03)	38.79* ± 0.177 (54.03)	30.58* ± 0.034 (38.94)
F value		12.1791	5.6470	4.0877	5.4991	6.9271
SE		0.7080	0.9592	0.5659	0.4428	0.4639
CD (*P* = 0.05)		2.0125	2.7265	1.6086	1.2586	1.3185
CD (*P* = 0.01)		2.6833	3.6353	2.1448	1.6782	1.7581

means ± SE of five replicates; values in parentheses indicate percent change over control; *significant at 1% level

of 64.5% on the fourth day in treated seedlings. Similarly, a significant and gradual increase in polyphenol oxidase activity (52%) was observed in UV-B treated seedlings throughout the study period although the increase was slightly lower than in peroxidase. Four days UV-B treatment also increased superoxide dismutase and phenylalanine ammonialyase activities by 54% and 38.9%, respectively, compared to the control.

DISCUSSION

In the present study, catalase activity was decreased by UV-B treatment. Catalase is the most efficient antioxidant enzyme, which protects plants by scavenging H_2O_2 (Vichnevetskaia & Roy 2001). However, it is susceptible to photoinactivation and degradation. It is also limited in its effectiveness by its selectively poor affinity for H_2O_2 (Foyer *et al*. 1997). In *Glycine max,* Singh (1996) observed that catalase activity was reduced with a simultaneous increase in peroxidase activity. Nandi *et al*. (1984) suggested that the inverted relationship between the two enzymes might be due to tetrameric molecules of catalase disintegrating *in vivo* into monomeric units with peroxidase activity.

Along with catalase activity, peroxidase activity is also an important component of the antioxidant stress system for scavenging H_2O_2. However, catalase changes H_2O_2 into O_2, whereas peroxidase decomposes H_2O_2 by oxidation of co-substances (Gaspar *et al*. 1991). In contrast to catalase in the present study, there was an increase of peroxidase in UV-B treated seedlings. This might be due to an increase in H_2O_2 production, probably as a result of induced superoxide dismutase activity during the treatment (Sharma *et al*. 1998). Further, peroxidases promote the utilisation of phenolic compounds as co-substrates (Otter & Polle 1994). This fact was confirmed by the accumulation

of higher phenolic contents observed in this study (data not shown). Gaspar *et al*. (1985) stated that increased basic peroxidase activity in response to stress decreases the indole acetic acid concentration and promotes acidic peroxidase synthesis. The increased trend in polyphenol oxidase activity in UV-B treated seedlings was observed in our studies and is responsible for the oxidation of phenolic compounds (Sheen & Calvert 1969). Namiki (1990) observed that polyphenols from dry bean may act as anti-oxidants to inhibit the formation of damaging free radicals.

Superoxide dismutase activity was also increased in UV-B treated seedlings after 4 days treatment. Karabourniotis *et al*. (1995) found that superoxide dismutase levels of wheat and maize were increased after exposing them to UV-B. Krizek *et al*. (1993) observed a definite pattern of superoxide dismutase under UV-B radiation in cucumber. The increase in the activity of superoxide dismutase observed in our study may be a consequence of production of O_2^- in leaves during UV-B radiation. Foyer *et al*. (1997) observed that superoxide dismutase provides protection from activated oxygen during periods of environmental stress.

In our experiments phenylalanine ammonialyase activity was increased. Similar results were observed in cucumber seedlings, where exposure to supplemental UV-B radiation caused a 78% increase in the activity of this enzyme (Beggs *et al*. 1985). Phenylalanine ammonialyase is an important enzyme in regulation of flavonoid biosynthesis and transcriptionally induced by UV-radiation (Hahlbrock & Scheel 1989). Dubey and Guruprasad (1999) suggested that the enhancement of phenylalanine ammonialyase is due to *de novo* synthesis of the enzyme and parallel to anthocyanin formation.

Enzymes such as peroxidase, polyphenol oxidase, phenylalanine ammonialyase and superoxide dismutase showed enhanced activity in UV-B treated seedlings and these enzymes might serve as acclimatisation mechanisms to scavenge the toxic free radicals of oxygen produced under stress condition. The results of the present work illustrates that in *Crotalaria juncea* L., UV-B radiation generates antioxidant substances that provide protection against UV-B radiation.

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References

Amrhein N., Zenk M.H. (1971): Untersuchungen zur Rolle der Phenylalanin-Ammonium-Lyase bei der Regulation der Flavonoidsynthese in Buchweizen (*Fagopyrum esculentum* Moench). Zeitschrift für Pflanzenphysiologie, **64**: 145–168.

Arora A., Sairam R.K., Srivastava G.C. (2002): Oxidative stress and antioxidative system in plants. Current Science, India, **82**: 1227–1238.

Beauchamp C.O., Fridovich I. (1971): Superoxide dismutase: Improved assays and an assay applicable to acrylamide gel. Analytical Biochemistry, **44**: 276–287.

Beggs C.J., Stolzer-Jehle A., Wellmann E. (1985): Isoflavonoid formation as an indicator of UV stress in bean (*Phaseolus vulgaris* L.) leaves. The significance of photo repair in assessing potential damage by increased solar UV-B radiation. Plant Physiology, **79**: 630–634.

Bowler C., Van Montagu M., Inze D. (1992): Superoxide dismutase and stress tolerance. Annual Review of Plant Physiology and Plant Molecular Biology, **43**: 83–116.

Brenda W.S. (2002): Biosynthesis of flavonoids and effects of stress. Current Opinion in Plant Biology, **5**: 218–223.

Coohill T.P. (1991): Stratospheric ozone depletion as it affects life on Earth – the role of ultraviolet action spectroscopy. In: Abrol Y.P., Wattal P.N., Gnanam A., Govindjee Ort D.R., Teramura A.H. (eds): Impact of Global Climatic Changes on Photosynthesis and Plant Productivity. Oxford & IBH Publishing Co., New Delhi: 3–21.

Dubey A., Guruprasad K.N. (1999): Introduction of anthocyanin synthesis by UV-B in *Sorghum bicolor* seedlings. Dependence on *de novo* synthesis of phenylalanine ammonialyase. Journal of Plant Biology, **26**: 225–229.

Foyer C., Noctor G., Morotagaudry J.F. (1997): Oxygen: Friend or foe for plants. Biofutur, **169**: 27–29.

Frederick J.E. (1990): Trends in atmospheric ozone and ultraviolet radiation: Mechanisms and observation for the northern hemisphere. Photochemistry and Photobiology, **51**: 757–763.

Gaspar T.H., Penel C., Castillo F.J., Greppin H. (1985): A two-step control of basic and acidic peroxidases and its significance for growth and development. Physiologia Plantarum, **64**: 418–423.

Gaspar T.H., Penel C., Hagega D., Greppin H. (1991): Peroxidases in plant growth, differentiation and development processes. In: Lobarzewski J., Gneppin H., Penel C., Gaspar T.H. (eds): Biochemical, Molecular and Physiological Aspects of Plant Peroxidases. University de Geneve, Switzerland: 249–250.

Hahlbrock K., Scheel D. (1989): Physiology and molecular biology of phenylpropanoid metabolism. Annual Review of Plant Physiology and Plant Molecular Biology, **40**: 367.

Iwanzik W., Tevini M., Dohnt G., Voss M., Weiss W., Graber P., Renger G. (1983): Action of UV-B radiation on photosynthetic primary reactions in spinach chloroplasts. Physiologia Plantarum, **58**: 401–407.

Karabourniotis G., Kotsabassidis D., Manetas Y. (1995): Trichome density and its protective potential against ultraviolet-B radiation on damage during leaf development. Canadian Journal of Botany, **73**: 376–383.

Krizek D.T., Kramer G.F., Upadhyaya A., Mirecki R.M. (1993): UV-B response of cucumber seedlings grown under metal halide and high pressure sodium/deluxe lamps. Physiologia Plantarum, **88**: 350–355.

Krupa S.V., Kickert N. (1989): The greenhouse effect: Impacts of ultraviolet-B (UV-B) radiation, carbondioxide (CO_2) and ozone (O_3) on vegetation. Environmental Pollution, **61**: 263–393.

Kumar K.B., Khan P.A. (1982): Peroxidase in excised ragi (*Eleusine coracana* cv. PR 202) leaves during senescence. Indian Journal of Experimental Botany, **20**: 412–416.

Maehly A.C., Chance B. (1959): The assay of catalase and peroxidase. In: Gilick D. (ed.): Methods of Biochemical Analysis. Vol. 1. International Science Publishers, Inc., New York: 357–425.

Namiki M. (1990): Antioxidants/antimutagens in food. Nutrition & Food Science, **29**: 273–300.

Nandi P.K., Agrawal M., Rao D.N. (1984): SO_2 induced enzymatic changes and ascorbic acid oxidation in *Oryza sativa.* Water, Air, and Soil Pollution, **21**: 25–32.

Nedunchezhian N., Kulandaivelu G. (1991): Effects of UV-B enhanced radiation on ribulose-1, 5-bisphosphate carboxylase in leaves of *Vigna sinensis* L. Photosynthetica, **25**: 431–435.

Nedunchezhian N., Kulandaivelu G. (1996): Effect of UV-B enhanced radiation and temperature on growth and photochemical activities in *Vigna unguiculata.* Biologia Plantarum, **38**: 205–214.

Negash L. (1987): Wavelength – dependence of stomatal closure by ultraviolet radiation in attached leaves of *Eragrostis tef*: action spectra under backgrounds of red and blue lights. Plant Physiology and Biochemistry, **25**: 753–760.

Otter T., Polle A. (1994): The influence of apoplastic ascorbate on the activities of cell-wall associated peroxidase and NADH-oxidases in needles of Norway spruce (*Picea abies* L.). Plant & Cell Physiology, **35**: 1231–1238.

Rao M.V., Gopinadhan P., Ormrod D.P. (1996): Ultraviolet-B and ozone-induced biochemicl changes in antioxidants enzymes of *Arabidopsis thaliana.* Plant Physiology, **110**: 125–136.

Ravindran K.C., Maheskumar N., Amirthalingam V., Ranganathan R., Chellappan K.P., Kulandaivelu G. (2001): Influence of UV-B supplemental radiation on growth and pigment content in *Suaeda maritima* L. Biologia Plantarum, **44**: 467–469.

Sharma P.K., Anand P., Sankhalkar S. (1998): Oxidative damage and changes in activities of antioxidant enzymes in wheat seedlings exposed to ultraviolet-B radiation. Current Science, **75**: 359–365.

Sheen S.J., Calvert J. (1969): Studies on polyphenol content, activities and isoenzymes of polyphenol oxidase and peroxidase during air-curing in three tobacco types. Plant Physiology, **44**: 199–104.

Singh A. (1996): Growth, physiological and biochemical responses of three tropical legumes to enhanced UV-B radiation. Canadian Journal of Botany, **74**: 135–139.

Tevini M., Teramura A.H. (1989): UV-B effects on terrestrial plants. Photochemistry and Photobiology, **50**: 479–487.

Tiwari A.K. (2004): Antioxidants: New-generation therapeutic base for treatment of polygenic disorders. Current Science, **86**: 1092–1102.

Tuckey J.W. (1953): The Problem of Multiple Comparisons. Princeton University Press, New Jersey.

Vichnevetskaia K.D., Roy D.N. (2001): Oxidative stress and antioxidative defence with an emphasis on plants antioxidants. Environmental Review, **7**: 31–51.

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Souhrn

Balakrishnan V., Venkatesan K., Ravindran K.C., Kulandaivelu G. (2005): **Ochranný mechanismus sazenic** *Crotalaria juncea* L. **proti působení UV-B záření**. Plant Protect. Sci., **41**: 115–120.

Jsou obavy, že některé antropogenní atmosférické polutanty (imise) mohou mít za následek globální snižování stratosférického ozonu. To by nepochybně mohlo zvýšit úroveň ultrafialového záření dopadajícího na povrch země. Takový nárůst slunečního ultrafialového záření však může mít významné biologické důsledky. Antioxidační

obranný systém rostlin zahrnuje mnoho různých antioxidačních molekul a enzymů. Zkoumali jsme roli aktivity enzymů antioxidantů v sazenicích *Crotalaria juncea* L., které byly vystaveny kontrolnímu (bez UV-B záření) a doplňkovému UV-B záření. Po šestihodinové aplikaci UV-B záření (10–16 h) ve čtyřech dnech nastal prudký útlum katalasové aktivity. Naproti tomu se však po čtyřech dnech aplikace UV-B záření (v porovnání s kontrolními sazenicemi) zvýšila aktivita peroxidasy, polyfenol oxidasy, superoxid dismutasy a fenylalanin amoniumlyasy. Takový nárůst aktivity těchto enzymů by mohl být adaptivním mechanismem minimalizace účinků UV-B záření.

Klíčová slova: *Crotataria juncea* L.; UV-B záření; antioxidant; katalasa

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