INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

EFFECT OF ELEVATED UV-B IRRADIATION ON THE NODULATION AND NITROGEN METABOLISM IN *SESBANIA GRANDIFLORA* (L.) PERS.

Sudaroli Sudha, J. & Rajendiran, K.
Department of Botany, K.M. Centre for Post Graduate Studies, Pondicherry – 605 008.

ABSTRACT
Nitrogen is the key substance apart from water that limits productivity of plants and the legume – *Rhizobium* symbiosis has become the major route for harnessing the gaseous nitrogen. CO_2 and other heat trapping gases which were dumped into the atmosphere by human activity, act like a blanket holding in heat around the earth. These gases increase in thickness warming the troposphere and cooling the stratosphere thereby indirectly depleting the ozone layer in addition to the direct method by ozone depleting substances (ODS). The depletion in the ozone layer allows enormous ultraviolet-B (UV-B) radiation into earth's surface affecting the growth of legumes and inhibiting biological nitrogen fixation. The present study is an attempt to assess the UV-B effects on nitrogen metabolism in the root and stem of *Sesbania grandiflora* (L.) Pers. The nodulation and nitrogen metabolism on 30 DAS (days after seed germination) of *Sesbania grandiflora* (L.) Pers. after exposure to supplementary UV-B radiation (2 hours daily @ 12.2 kJ m^{-2} d^{-1}; ambient = 10 kJ m^{-2} d^{-1}), were monitored. UV-B stress decreased the protein and amino acid contents of *Sesbania grandiflora* (L.) Pers. in the leaves by 37 and 31 % respectively and reduced nitrate and nitrite by 13 and 32 % in the leaves, by 15 and 16 % in the stem nodules and by 17 and 21 % in the root nodules. UV-B exposure suppressed NRA (nitrate reductase activity) by 38 % in leaves, 17 % in stem nodules and 21 % in nodules. Nodulation was suppressed by UV-B as the number of stem nodules (45 %), number of root nodules (51 %) fresh mass of stem nodules (38 %) and root nodules (21 %) were far below controls. UV-B stress also inhibited nitrogenase enzyme activity by 25 % in roots, by 68 % in stem nodules and by 62 % in stem nodules. Present study indicates that any further increase in depletion of ozone layer might enhance UV-B stress on crop plants thereby depressing the symbiotic nitrogen fixation in legumes and affecting sustainable food production.

KEY WORDS: Ultra violet-B stress, global warming, *Sesbania grandiflora*, stems nodules, root nodules, nitrogen metabolism.

INTRODUCTION

Depletion of ozone layer has become an insurmountable environmental problem in the recent past. It threatens to continue so as the green house gases around the globe increases in thickness and the heat that normally would escape the troposphere and enter the stratosphere no longer does so, leaving the stratosphere cooler. Colder than normal temperatures in this layer enhances ozone depletion, which is considered as an indirect effect of global warming in addition to the direct depletion by the ozone depleting substances (ODS). As a result, the UV-B fluence is bound to increase, affecting plants, animals and human beings, and in the long run, the ecosystems too. An increase in the flux of ultraviolet-B (UV-B) radiation (280 - 320 nm) is an important atmospheric stress and is detrimental to plant growth and development (Caldwell *et al.,* 1998, Rajendiran and Ramanujam 2000, Rajendiran and Ramanujam 2003 and Rajendiran and Ramanujam 2004). At the metabolism level, it severely inhibits photosynthesis (Caldwell *et al*., 1998, Kulandaivelu and Lingakumar 2000) and hampers nodulation and nitrogen fixation (Balakumar *et al.,* 1993, Rachel and Santhaguru 1999, Rajendiran and Ramanujam 2006) in sensitive plants. Although plants generally develop tolerance to increases in UV-B flux, the objective of the present study was to find out the extent of damage caused by supplementary UV-B on nodulation and nitrogen metabolism of *Sesbania grandiflora* (L.) Pers., a root and stem nodulating plant and an important member of green manuring in agriculture.

MATERIALS & METHODS

Sesbania grandiflora (L.) Pers. plants were grown in pot culture in the naturally lit greenhouse (day temperature maximum 38 ± 2 ºC, night temperature minimum 18 ± 2 ºC, relative humidity 60 ± 5 %, maximum irradiance (PAR) 1400 µmol m-2 s-1, and photoperiod 12 to 14 h). Supplementary UV-B radiation was provided in UV garden by three UV-B lamps (*Philips TL20W/12 Sunlamps*, The Netherlands), which were suspended horizontally and wrapped with cellulose diacetate filters (0.076 mm) to filter UV-C radiation (< 280 nm). UV-B exposure was given for 2 h daily from 10:00 to 11:00 and 15:00 to 16:00 starting from the 5th day after sowing. Plants received a biologically effective UV-B dose (UV-BBE) of 12.2 kJ m-2 d-1 equivalents to simulated 20 % ozone depletion at Pondicherry (12º2'N, India). The control plants, grown under natural solar radiation, received UV-BBE 10 kJ m-2 d-1. The seedlings (10 days old) in each pot were inoculated with 200 mg of the commercial preparation of *Rhizobium* (cowpea strain) inoculum suspended in 1 cm^3 of water and poured on the surface of the soil as suggested by Shriner and Johnston (1981). Ten plants from each treatment and control were carefully uprooted from the soil at 30 DAS (days after seed germination) and the number and fresh mass of both

the stem and root nodules were recorded. The nitrate and nitrite contents, nitrogenase and nitrate reductase activity of the leaf, root, root nodules and stem nodules were recorded at 30 DAS, since nodulation was at its peak level during this period. The biochemical estimations were made from the compound leaves at 30 DAS. The amino acid content was determined by the method of Moore and Stein (1948). Soluble proteins were estimated using Folin phenol reagent method (Lowry *et al*., 1951). Nitrate and nitrite contents were determined using naphthylamine salt-mixture (Woolley *et al*., 1960). *In vivo* NRA was assayed by the method of Jaworski (1971) with suitable modifications (Muthuchelian *et al*., 1993). Nodular nitrogenase activity was determined by the acetylene reduction technique (Stewart *et al*., 1967). The values were analysed by Tukey's multiple range test (TMRT) at 5 % level of significance (Zar, 1984).

RESULT AND DISCUSSION

Elevated UV-B radiation decreased the protein and amino acid contents of *Sesbania grandiflora* (L.) Pers. in the leaves by 37 and 31 % respectively (Table 1, Fig. 1). Reductions in soluble protein and amino acid contents of leaves are features of UV-B stress (Tevini *et al*., 1981, Vu *et al*.,1981, Rajendiran and Ramanujam, 2006). Unstressed plants accumulated more nitrate and nitrite both in the stem and the root nodules (Table 1, Fig. 1). However UV-B stressed plants showed reduction by 13 and 32 % in the leaves, by 15 and 16 % in the stem nodules and by 17 and 21 % in the root nodules (Table 1, Fig. 1). Ghisi *et al.* (2002) in barley and Rajendiran and Ramanujam (2006) in *Vigna radiata* observed significant reductions in the activities of nitrate reductase and glutamine synthetase, not only in the UV-B receiving leaves but also in the root system. Chimphango *et al*. (2003) found no adverse effect of elevated UV-B radiation on growth and symbiotic function of *Lupinus luteus* and *Vicia atropurpurea* plants. UV-B exposure suppressed NRA by 38 % in leaves, 17 % in stem nodules and 21 % in nodules. Similar results of decreased values of NRA after exposure to UV-B radiation in comparison with control seedlings were reported in the leaves and roots of *Zea mays* L. (Quaggiotti *et al.* 2004).

TABLE 1. Changes in number and fresh mass of nodules (g) per root system and per shoot system, contents of proteins [mg g^{-1}(f.m.)], amino acids, nitrates and nitrites [mg g^{-1}(d.m.)], and the activities of nitrate reductase, NRA [µmol(NO_2-) kg^{-1}(f.m.) s^{-1}] and nitrogenase, N_2-ase [µmol(ethylene reduced) g^{-1}(f.m.) s^{-1}] in the 30 DAS leaves, roots and nodules of *Sesbania grandiflora* (L.) Pers. exposed to supplementary UV-B radiation. Means followed by different letters are significantly different at $P = 0.05$, $n = 10$.

Organ	Parameter	Control	UV - B
Leaf	Protein	17.02b	10.66a
	Amino acid	25.02b	17.18a
	Nitrate	3.32b	2.87a
	Nitrite	0.31b	0.17a
	NRA	1.57b	0.96a
Stem nodule	Nodule number	33.00b	18.00a
	Nodule fresh mass per stem	0.25b	0.11a
	Nitrate	2.44a	3.05b
	Nitrite	0.36b	0.30a
	NRA	2.18b	1.79a
	N_2-ase	29.00b	11.00a
Root nodule	Nodule number	38.3b	18.6a
	Nodule fresh mass per root	42.00b	26.00a
	Nitrate	4.46b	3.68a
	Nitrite	0.32b	0.25a
	NRA	1.58a	1.99b
	N_2-ase	31.48b	10.00a
Root	N_2-ase	0.40b	0.30a

Such a decline in NRA was found related to changes in the protein synthesis and degradation (Bardizick *et al*., 1971) or inactivation of the enzyme (Plaut, 1974). However Marek, *et al.* (2008) in *Pinus sylvestris* L. needle reported an enhancement of NRA after exposure to UV-B irradiance. The nitrate accumulation consequent to UV-B induced inhibition of NRA was observed by Guerrero *et al*. (1981) but was not confirmed by this study. Such a disparity occurred in UV-B stressed *Vigna unguiculata* also (Balakumar *et al*., 1993). According to Ghisi *et al*. (2002), nitrate content of neither the leaf nor root was influenced by elevated UV-B. Nodulation was inhibited severely by UV-B as the number of stem nodules (45 %), number of root nodules (51 %), size and fresh mass of stem nodules (38 %) and root nodules (21 %) were far below controls. In contrast, nodulation and nitrogen fixation in three legumes *viz*. *Vigna unguiculata*, *Glycine max* and *Phaseolus mungo* were not affected by exposure to 32 and 62 % above ambient UV-B (Samson *et al.,* 2004). UV-B stress inhibited nitrogenase enzyme activity by 25 % in roots, by 68 % in stem nodules and by 62 % in stem nodules. Hence, UV-B which continues to be a critical environmental stress apart from affecting the metabolism of the aerial parts of the crops also alters the vital functions of the root system thereby adversely

suppressing the nitrogen metabolism and causing a threat to food and nutrient security.

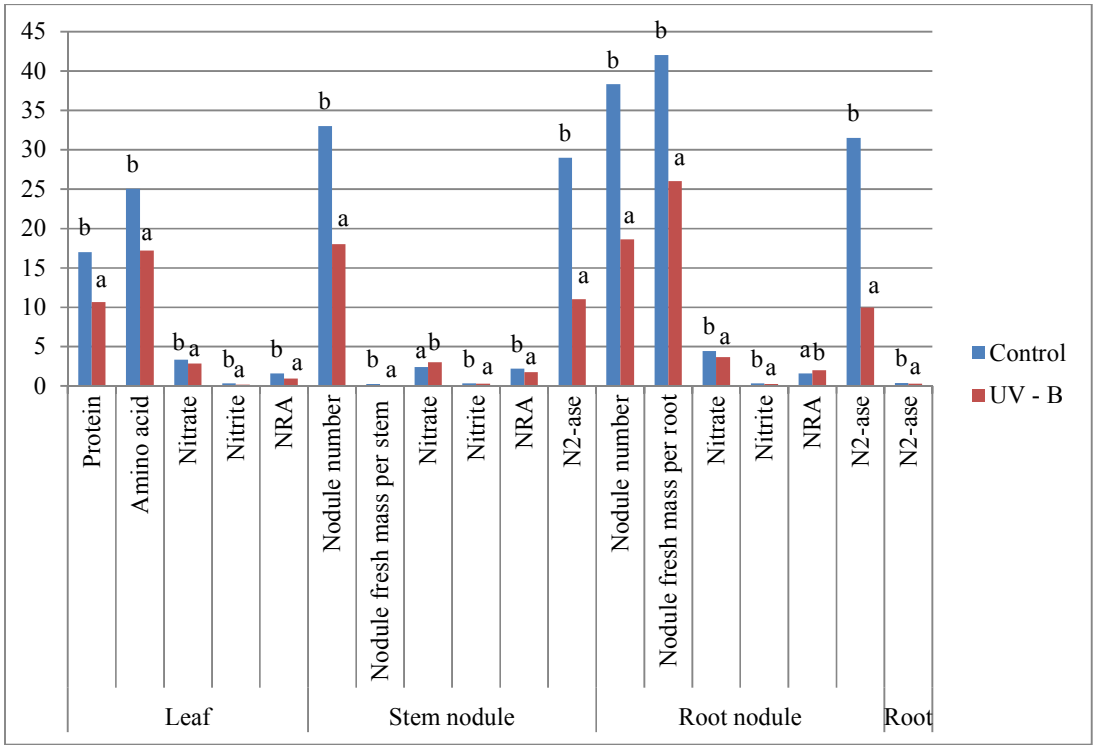

FIGURE 1. Changes in number and fresh mass of nodules (g) per root system and per shoot system, contents of proteins [mg g^{-1}(f.m.)], amino acids, nitrates and nitrites [mg g^{-1}(d.m.)], and the activities of nitrate reductase, NRA [μmol(NO_2-) kg^{-1}(f.m.) s^{-1}] and nitrogenase, N_2-ase [μmol(ethylene reduced) g^{-1}(f.m.) s^{-1}] in the 30 DAS leaves, roots and nodules of *Sesbania grandiflora* (L.) Pers. exposed to supplementary UV-B radiation. Means followed by different letters are significantly different at $P = 0.05$, $n = 10$.

REFERENCES

Balakumar, T., Vincent, V. H. B., Paliwal, K. (1993) On the interaction of UV-B radiation (280-315 nm) with water stress in crop plants. - Physiol. Plant. **87**: 217-222.

Bardizick, J. M., Marsh, H.V., Havis, J. R. (1971) Effects of water stress on the activities of three enzymes in maize seedlings. - Plant Physiol. **47**: 828-831.

Caldwell, M. M., Bjorn, L.O., Bornman, J.F., Flint, S.D., Kulandaivelu, G., Teramura, A. H., Tevini, M. (1998) Effects of increased solar ultraviolet radiation on terrestrial ecosystem. - Photochem. Photobiol. **46**: 40-52.

Chimphango, S. B., Musil, C. F., Dakora, F. D. (2003) Response of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea* to ultraviolet-B radiation. - J.exp. Bot. **54**: 1771-1784.

Ghisi, R., Trentin, A. R., Masi, A., Ferretti, M. (2002) Carbon and nitrogen metabolism in barley plants exposed to UV-B radiation. - Physiol. Plant. **116**: 200-205.

Guerrero, M. G., Veg, J. M., Losada, M. (1981) The assimilatory nitrate reducing system and its regulation. - Annu. Rev. Plant Physiol. **32**: 169-294.

Jaworski, E. G. (1971) Nitrate reductase in intact plant tissue. – Biochem. biophys. Res. Commun. **43**: 1274-1279.

Marek, K., Jerzy, S., Heli, K., Françoise, M., Marja-Liisa S., Kaisa, L., Minna, T. (2008) Influence of solar UV radiation on the nitrogen metabolism in needles of Scots pine (*Pinus sylvestris* L.) Environmental Pollution.**156 (3):** 1105-1111.

Kulandaivelu, G., Lingakumar, K. (2000) Molecular targets of UV-B radiation in the photosynthetic membranes. - In: Yunus, M., Pathre, U., Mohanty, P. (ed.): Probing photosynthesis, Mechanisms, Regulation and Adaptation. Pp. 364-378. Taylor and Francis Publications, New York.

Lowry, O. H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. - J. biol. Chem. **193**: 265-275.

Moore, S., Stein, W.H. (1948) Photometric method for use in the chromatography of amino acids. - J. biol. Chem. **176**: 367-388.

Muthuchelian, K., Nedunchezhian, N., Kulandaivelu, G. (1993) Effect of simulated acid rain on 14CO_2 fixation, ribulose-1,5- bisphosphate carboxylase and nitrate and

nitrite reductase in *Vigna sinensis* and *Phaseolus mungo*. - Photosynthetica **28**: 361-367.

Plaut, Z. (1974) Nitrate reductase activity of wheat seedlings during exposure to and recovery from water stress and salinity. - Physiol. Plant. **30**: 212-217.

Quaggiotti, S., Trentin, A. R., Vecchia, F. D. and Ghisi, R. (2004) Response of maize (*Zea mays* L.) nitrate reductase to UV-B radiation. Plant Sci. **167:** 107-116.

Rachel, D., Santhaguru, K. (1999) Impact of UV-B irradiation on growth, nodulation and nitrate assimilation in *Vigna mungo* L. and *Vigna radiata* L. Wilczek. - In: Srivastava, G. C., Singh, K., Pal, M. (ed.): Plant Physiology for Sustainable Agriculture, Pointer Publishers, Jaipur. Pp. 294-300.

Rajendiran, K., Ramanujam, M. P. (2000) Efficacy of triadimefon treatment in ameliorating the UV-B stress in green gram. - In: Khan, M. (ed.): National Symposium on Environmental Crisis and Security in the New Millennium. Pp. 41-42. National Environmental Science Academy, New Delhi.

Rajendiran, K., Ramanujam, M. P. (2003) Alleviation of ultraviolet-B radiation-induced growth inhibition of green gram by triadimefon. - Biol. Plant. **46**: 621-624.

Rajendiran, K., Ramanujam, M.P. (2004) Improvement of biomass partitioning, flowering and yield by triadimefon in UV-B stressed *Vigna radiata* (L.) Wilczek. - Biol. Plant.**48**: 145-148.

Rajendiran, K., Ramanujam, M.P. (2006) Interactive effects of UV-B irradiation and triadimefon on nodulation and nitrogen metabolism in *Vigna radiata* plants. Biologia Plantarum. 50 (4): 709-712.

Samson, B.M., Chimphango, F.B., Musil, C.F., Dakora, F.D. (2004) Effects of UV-B radiation on plant growth, symbiotic function and concentration of metabolites in three tropical grain legumes. - Functional Plant Biol. **30**: 309-318.

Shriner, D.S., Johnston, J.W. (1981) Effects of simulated acidified rain on nodulation of leguminous plants by *Rhizobium* spp. - Environ. exp. Bot. **21**: 199-209.

Stewart, W.D.P., Fitzgerald, G.P., Burris, R.H. (1967) *In situ* studies on nitrogen fixation using the acetylene reduction technique. - Proc. nat. Acad. Sci. USA **58**: 2071-2078.

Tevini, M., Iwanzik, W., Thoma, U. (1981) Some effects of enhanced UV-B radiation on the growth and composition of plants. - Planta **153:** 388-394.

Vu, C.V., Allen, L.H., Garrard, L.A. (1981) Effects of supplementary UV-B radiation on growth and leaf photosynthetic reactions of soybean (*Glycine max*). - Physiol. Plant. **52**: 353-362.

Woolley, J.T., Hicks, G.P., Hageman, R.H. (1960) Rapid determination of nitrate and nitrite in plant material. - J. agr. Food Chem. **8**: 481-482.

Zar, J.H. (1984) Bio-statistical Analysis. - Prentice-Hall, Englewood Cliffs.