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Research Article

SUPPLEMENTARY ULTRAVIOLET-B INDUCED REDUCTION ON NODULATIONAND NITROGEN METABOLISM IN HYACINTH BEAN

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ABSTRACT
Symbiotic nitrogen fixation occurs in plants that harbour nitrogen-fixing bacteria within their tissues. The best-studied example is the association between legumes and bacteria in the genus *Rhizobium.* Each of these is able to survive independently (soil nitrates must then be available to the legume), but life together is clearly beneficial to both. Only together can nitrogen fixation take place. Human activity has dumped CO_2 and other heat trapping gases into the atmosphere which acts like a blanket holding in heat around the earth. When these gases increase in thickness, they are 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 amount of ultraviolet-B(UV-B) radiation into earth's surface, thus 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 leaves, roots and nodules of hyacinth bean, *Lablab purpureus* L. var. Rongai. The nodulation and nitrogen metabolism on 30 and 45 DAS (days after seed germination) of hyacinth bean 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 *Lablab purpureus* L. var. Rongai. in the leaves by 40 to 42 % and 32 to 35 % respectively and reduced nitrate and nitrite by 26 to 35 % and 37 to 44 % in the leaves and by 44 to 57 % and 46 to 50 % in the root nodules. UV-B exposure suppressed NRA (nitrate reductase activity) by 32 to 42 % in leaves and 32 to 37 % in nodules. Nodulation was suppressed by UV-B as the number of root nodules (35 to 46 %) and their fresh mass (32 to 39 %) were far below controls. UV-B stress also inhibited nitrogenase enzyme activity by 46 to 55 % in roots and by 40 to 47 % in root nodules. Further accumulation of heat-trapping gases in the atmosphere will enhance the depletion of ozone layer, increasing the UV-B stress in legumes, thereby affecting the symbiotic nitrogen fixation.

Keywords: *Global Warming, Ultraviolet-B Stress, Lablab purpureus, Root Nodules, Nitrogen Metabolism*

INTRODUCTION
Without the layer of ozone in the stratosphere to protect us from excessive amounts ofUV-B radiation, life as we know would not exist. Ozone layer depletion threatens to continue so as the green house gases around the globe increase in thickness, and the heat that normally would escape the troposphere and enter the stratosphere no longer does so, leaving the stratosphere cooler. Temperatures lower than normal in this layer will enhance ozone depletion, which is considered as an indirect effect of global warmingin addition to the direct depletion by the ozone depleting substances (ODS). Scientific concern over ozone depletion in the upper atmosphere has prompted extensive efforts to assess the potential damage to life on earth due to increased levels of UV-B radiation. An elevation in the flux of ultraviolet-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; Rajendiran and Ramanujam, 2004; Kokilavani and Rajendiran, 2013.). 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;Sudaroli Sudha and Rajendiran 2013a, 2013b) in sensitive plants. Although plants generally develop tolerance to increases in UV-B flux, the objective of the present study was to assess the extent of damage

caused by UV-B on nodulation and nitrogen metabolism of *Lablab purpureus* L. var. Rongai, a dual purpose legume which can be sown with summer grass crops to provide a mixed forage crop system.

MATERIALS AND METHODS

Lablab purpureus L. var. Rongai, 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}, 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 ($UV\text{-}B_{BE}$) of 12.2 kJ m^{-2} d^{-1} equivalent to a simulated 20 % ozone depletion at Pondicherry (12º2'N, India). The control plants, grown under natural solar radiation, received $UV\text{-}B_{BE}$ 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 and 45 DAS (days after seed germination) and the number and fresh mass of root nodules were recorded. The nitrate and nitrite contents, nitrogenase and nitrate reductase activity of the leaf, root and root nodules were recorded at 30 and 45 DAS, since nodulation was at its peak level during this period. The biochemical estimations were made from the compound leaves at 30 and 45 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).

RESULTS AND DISCUSSION

The protein and amino acid contents of UV-B stressed *Lablab purpureus* L. var. Rongai, decreased by 40 to 42 % and 32 to 35 % respectively in the leaves (Table 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 and Sudaroli Sudha and Rajendiran 2013a, 2013b). Plants grown in controlled condition accumulated more nitrate and nitrite in the root nodules while UV-B stressed plants showed reduction in nitrate and nitrite by 26 to 35 % and 37 to 44 % in the leaves and by 44 to 57 % and 46 to 50 % in the root nodules respectively (Table 1). Ghisi *et al.*,(2002) in barley, Rajendiran and Ramanujam (2006) in *Vigna radiata* (L.) Wilczek., Sudaroli Sudha and Rajendiran (2013a) in *Sesbania grandiflora* (L.) Pers. and Sudaroli Sudha and Rajendiran (2013b) in *Vigna unguiculata* (L.) Walp. c.v. BCP-25 have reported significant reductions in nitrate reductase and glutamine synthetase activities both in the UV-B exposed leaves as well as in the root system. However there was noadverse effect of elevated UV-B irradiation on growth and symbiotic nitrogen fixation of *Lupinus luteus* and *Vicia atropurpurea* plants (Chimphango *et al.,* 2003). UV-B radiation reduced NRA by 32 to 42 % in leaves and 32 to 37 % in nodules. The leaves as well as the roots of *Zea mays* L. (Quaggiotti *et al.,* 2004), *Vigna radiata* (L.) Wilczek. (Rajendiran and Ramanujam 2006) *Sesbania grandiflora* (L.) Pers. (Sudaroli Sudha and Rajendiran 2013a) and *Vigna unguiculata* (L.) Walp. c.v. BCP-25 (Sudaroli Sudha and Rajendiran 2013b) showed decreased values of NRA after UV-B irradiancewhen compared with control seedlings. According to Bardizick *et al.,* (1971), Plaut (1974) and Rajendiran and Ramanujam (2006), a decrease in NRA was found related to changes in the protein synthesis and degradation or inactivation of the enzyme. However Marek, *et al.,* (2008) in *Pinus sylvestris* L. needle reported an enhancement of NRA after exposure to UV-B exposure. Guerrero *et al.,* (1981) observed an accumulation of the nitrate consequent to UV-B induced inhibition of NRA, but was not confirmed by this study. Balakumar *et al.,* (1993) also

reported such a disparity in UV-B and water stressed *Vigna unguiculata*. 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 root nodules (35 to 46 %), size and fresh mass of root nodules (32 to 39 %) were drastically reduced under controls. In contrast, nodulation and nitrogen fixation in three tropical grain legumes 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 46 to 55 % in roots and by 40 to 47 % in root nodules. Even though the same trend of reduction in all the parameters was observed in 30 as well as 45 DAS UV-B exposed plants, suppression of nitrogen metabolism was found to be severe in 30 DAS. In conclusion, UV-B radiation which was proved to be detrimental to the metabolism of the aerial parts of the plants also disturbs the vital functions of the root system thereby adversely affecting the symbiotic nitrogen fixation in legumes.

Table 1:Changes in number and fresh mass (g) of nodules per root 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 and 45 DAS (days after seed germination) leaves, roots and nodules of *Lablab purpureus*L. var. Rongai 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	
		30DAS	45DAS	30DAS	45DAS
Leaf	Protein	19.22b	21.46b	11.58a	12.39a
	Amino acid	25.68b	28.65b	16.68a	19.28a
	Nitrate	5.67b	6.54b	3.67a	4.79a
	Nitrite	0.25b	0.29b	0.14a	0.18a
	NRA	1.72b	2.12b	1.16a	1.21a
Root Nodule	Nodule Number	20.6b	30.6b	13.3a	16.3a
	Nodule Fresh Mass per root	0.23b	0.28b	0.14a	0.19a
	Nitrate	4.12b	5.82b	2.27a	2.46a
	Nitrite	0.42b	0.49b	0.21a	0.26a
	NRA	2.29b	2.76b	1.55a	1.73a
	N_2-ase	23.44b	26.87b	13.87a	14.22a
Root	N_2-ase	0.47b	0.52b	0.21a	0.28a

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REFERENCES
Balakumar T, Vincent VHB, Paliwal K (1993). On the interaction of UV-B radiation (280-315 nm) with water stress in crop plants. *Physiologia Plantarum* **87** 217-222.
Bardizick JM, Marsh HV and Havis JR (1971). Effects of water stress on the activities of three enzymes in maize seedlings. *Plant Physiology* **47** 828-831.
Caldwell MM, Bjorn LO, Bornman JF, Flint SD, Kulandaivelu G, Teramura AH and Tevini M (1998). Effects of increased solar ultraviolet radiation on terrestrial ecosystem.*Photochemistry and Photobiology* **46** 40-52.
Chimphango SB, Musil CF and Dakora FD (2003). Response of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea* to ultraviolet-B radiation. *Journal of Experimental Botany* **54** 1771-1784.
Ghisi R, Trentin AR, Masi A and Ferretti M (2002). Carbon and nitrogen metabolism in barley plants exposed to UV-B radiation. *Physiologia Plantarum* **116** 200-205.

Guerrero MG, Veg JM and Losada M (1981). The assimilatory nitrate reducing system and its regulation. *Annual Review of Plant Physiology* **32** 169-294.

Jaworski EG (1971). Nitrate reductase in intact plant tissue. *Biochemical and Biophysical Research Communications* **43** 1279.

Kokilavani V and Rajendiran K (2013). Ultraviolet-B induced changes in the leaf epidermal and anatomical characteristics of *Vigna mungo* L.var. KM-2. *International Journal of Science and Nature***5**(1) 126-130.

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

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry* **193** 265-275.

Marek K, Jerzy S, Heli K, Françoise M, Marja-Liisa S, Kaisa L and 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.

Moore S and Stein WH (1948). Photometric method for use in the chromatography of amino acids. *The Journal of Biological Chemistry* **176** 367-388.

Muthuchelian K, Nedunchezhian N and 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. *Physiologia Plantarum* **30** 212-217.

Quaggiotti S, Trentin AR, Vecchia FD and Ghisi R (2004). Response of maize (*Zea mays* L.) nitrate reductase to UV-B radiation. *Plant Science* **167** 107-116.

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

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

Rajendiran K and Ramanujam MP (2003). Alleviation of ultraviolet-B radiation-induced growth inhibition of green gram by triadimefon. *Biologia Plantarum* **46** 621-624.

Rajendiran K and Ramanujam MP (2004). Improvement of biomass partitioning, flowering and yield by triadimefon in UV-B stressed *Vigna radiata* (L.) Wilczek. *Biologia Plantarum* **48** 145-148.

Rajendiran K and Ramanujam MP (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 BM, Chimphango FB, Musil CF and Dakora FD (2004). Effects of UV-B radiation on plant growth, symbiotic function and concentration of metabolites in three tropical grain legumes. *Functional Plant Biology* **30** 309-318.

Shriner DS and Johnston JW (1981). Effects of simulated acidified rain on nodulation of leguminous plants by *Rhizobium* spp.*Environmental and Experimental Botany* **21** 199-209.

Stewart WDP, Fitzgerald GP and Burris RH (1967). *In situ* studies on nitrogen fixation using the acetylene reduction technique. *Proceedings of the National Academy of Sciences USA* **58** 2071-2078.

Sudaroli Sudha J and Rajendiran K (2013a). Effect of elevated UV-B irradiation on the nodulation and nitrogen metabolism in *Sesbania grandiflora* (L.) Pers. *International Journal of Science and Nature***4**(4) 664-667.

Sudaroli Sudha J and Rajendiran K (2013b). Effect of elevated UV-B irradiation on the nodulation and nitrogen metabolism in *Vigna unguiculata* (L.) Walp. c.v. BCP-25. *International Journal of Food, Agriculture and Veterinary Sciences***3**(3) 77 - 81.

Tevini M, Iwanzik W and Thoma U (1981). Some effects of enhanced UV-B radiation on the growth and composition of plants. *Planta* **153** 388-394.
Vu CV, Allen LH and Garrard LA (1981). Effects of supplementary UV-B radiation on growth and leaf photosynthetic reactions of soybean (*Glycine max*). *Physiologia Plantarum* **52** 353-362.
Woolley JT, Hicks GP and Hageman RH (1960). Rapid determination of nitrate and nitrite in plant material. *Journal of Agricultural and Food Chemistry* **8** 481-482.
Zar JH (1984). *Bio-Statistical Analysis* (Prentice-Hall, Englewood Cliffs).