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EFFECT OF SUPPLEMENTARY ULTRAVIOLET-B RADIATION ON NODULATION AND NITROGEN METABOLISM IN *LABLAB PURPUREUS* L. VAR. GOLDY

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ABSTRACT
Solar UV radiation, climate and other drivers of global change are undergoing significant changes, and models forecast that these changes will continue for the remainder of this century. CO_2 and other heat trapping gases which are being pumped into the atmosphere by human, act like a blanket holding hotness around the earth. Increase in volume of these gases will warm the troposphere but cool 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, 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. Goldy. 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. Goldy in the leaves by 33 to 41 % and 23 to 31 % respectively and reduced nitrate and nitrite by 27 to 36 % and 17 to 30 % in the leaves and by 37 to 47 % and 34 to 50 % in the root nodules. UV-B exposure suppressed NRA (nitrate reductase activity) by 28 to 47 % in leaves and 27 to 50 % in nodules. Nodulation was suppressed by UV-B as the number of root nodules (43 %) and their fresh mass (30 to 47 %) were far below controls. UV-B stress also inhibited nitrogenase enzyme activity by 28 to 44 % in roots and by 44 to 46 % in root nodules. Any increase in depletion of ozone layer in future might enhance UV-B stress on crop plants, thereby depressing the symbiotic nitrogen fixation in legumes and affecting sustainable food production.

KEY WORDS: 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 of UV-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. Decrease in temperature 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). 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. 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 and 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 find out the extent of damage caused by supplementary UV-B on nodulation and nitrogen metabolism of *Lablab purpureus* L. var. Goldy, a dual purpose legume which can be sown with summer grass crops to provide a mixed forage crop system.

MATERIALS & METHODS

Lablab purpureus L. var. Goldy (hyacinth bean) 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-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-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 both the stem and 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).

RESULT & DISCUSSION

The protein and amino acid contents of UV-B stressed *Lablab purpureus* L. var. Goldy, decreased by 33 to 41 % and 23 to 31 % 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).

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. Goldy 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	17.33b	21.25b	10.27a	11.56a
	Amino acid	22.57b	24.69b	15.46a	17.32a
	Nitrate	5.34b	5.97b	3.43a	3.86a
	Nitrite	0.23b	0.28b	0.16a	0.19a
	NRA	2.34b	2.85b	1.24a	1.68a
Root Nodule	Nodule Number	21.5b	27.5b	12.3a	15.3a
	Nodule Fresh Mass per root	0.23b	0.28b	0.12a	0.16a
	Nitrate	3.27b	3.85b	1.71a	2.05a
	Nitrite	0.26b	0.33b	0.13a	0.17a
	NRA	2.56b	2.75b	1.28a	1.87a
	N_2-ase	21.12b	23.26b	11.28a	11.64a
Root	N_2-ase	0.38b	0.42b	0.21a	0.27a

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 27 to 36 % and 17 to 30 % in the leaves and by 37 to 47 % and 34 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 Chimphango *et al*. (2003) did not find any adverse effect of elevated UV-B radiation on growth and symbiotic function of *Lupinus luteus* and *Vicia atropurpurea* plants. UV-B irradiation suppressed NRA by 28 to 47 % in leaves and 27 to 50 % 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 irradiance when 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 (43 %), size and fresh mass of root nodules (30 to 47 %) were drastically reduced under controls. In contrast, nodulation and nitrogen fixation in three tropical grain legumes were not affected by exposure to elevated UV-B radiation (Samson *et al.*, 2004). UV-B stress inhibited nitrogenase enzyme activity by 28 to 44 % in roots and by 44 to 46 %

in root nodules. The UV-B exposed plants on 30 as well as 45 DAS recorded a general reduction in all the parameters compared to unstressed plants. However, the suppression of nitrogen metabolism was found to be severe in stressed plants on 30 DAS. The present study provided direct evidence for the inhibition of essential functions of the root system by supplementary UV-B, which was earlier believed to alter only the metabolism of aerial parts of the plants.

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