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Research Article

EFFECT OF ELEVATED UV-B IRRADIATION ON THE NODULATION AND NITROGEN METABOLISM IN *VIGNA UNGUICULATA* (L.) WALP. CV. BCP-25

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ABSTRACT
Nitrogen an essential component of the proteins that build cell materials and plant tissue comes from fertilizer application but the legumes can convert atmospheric nitrogen to plant-available forms through a symbiotic biological process involving *Rhizobium* bacteria and the plant roots. 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. 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 *Vigna unguiculata* (L.) Walp cv. BCP-25. The nodulation and nitrogen metabolism on 30 DAS (days after seed germination) of *Vigna unguiculata* (L.) Walp cv. BCP-25 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 *Vigna unguiculata* (L.) Walp cv. BCP-25 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. 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: *Global Warming, Ultra Violet-B Stress, Vigna unguiculata, Root Nodules, Nitrogen Metabolism*

INTRODUCTION
Ozone layer depletion has become an unconquerable environmental trouble 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 ultraviolet-B (UV-B) radiation is bound to elevate, affecting plants, animals and human beings and the ecosystems. 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). 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 AND METHODS

Vigna unguiculata (L.) Walp cv. BCP-25 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-BBE) 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-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, stem nodules, root and root 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).

RESULTS AND DISCUSSION

The protein and amino acid contents of UV-B stressed *Vigna unguiculata* (L.) Walp cv. BCP-25 decreased by 43 and 31 % respectively in the leaves (Table 1). According to Tevini *et al.,* (1981), Vu *et al.,* (1981), Rajendiran and Ramanujam (2006) reductions in soluble protein and amino acid contents of leaves are features of UV-B stress. Plants grown in controlled condition accumulated more nitrate and nitrite in the root nodules (Table 1). On the other hand UV-B stressed plants showed reduction by 17 and 33 % in the leaves and by 30 and 31 % in the root nodules (Table 1). Ghisi *et al.,* (2002) in barley and Rajendiran and Ramanujam (2006) in *Vigna radiata* have reported significant reductions in nitrate reductase and glutamine synthetase activities both in the UV-B receiving leaves as well as in the root system. However 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 irradiation suppressed NRA by 47 % in leaves and 31 % in nodules. Both the leaves and roots of *Zea mays* L. showed decreased values of NRA after exposure to UV-B radiation in comparison with control seedlings (Quaggiotti *et al.,* 2004). 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. 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. Such a disparity was also reported by Balakumar *et al.,* (1993) 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 %), size and fresh mass of root nodules (45 %) 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 19 % in roots and by 61 % in root nodules.

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 d leaves, roots and nodules of *Vigna unguiculata* exposed to supplementary UV-B radiation, aqueous triadimefon (TRIAD, 20 mg dm-3) and their combinations. Means followed by different letters are significantly different at $P = 0.05$, $n = 10$

Organ	Parameter	Control	UV - B
Leaf	Protein	19.06b	10.82a
	Amino acid	26.24b	18.03a
	Nitrate	5.42b	4.46a
	Nitrite	0.27b	0.18a
	NRA	2.06b	1.08a
Root Nodule	Nodule Number	34.5b	22.3a
	Nodule Fresh Mass per root	0.20b	0.11a
	Nitrate	4.24b	2.97a
	Nitrite	0.29b	0.20a
	NRA	2.47b	1.88a
	N_2-ase	32.00b	12.20a
Root	N_2-ase	0.36b	0.29a

To conclude, UV-B which threatens to be a dangerous environmental stress not only affects the metabolism of the aerial parts of the plants but also disturbs the essential functions of the root system thereby adversely affecting the nitrogen metabolism and creating a threat to nutrient content of the food grains.

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