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***Research Article***

# EFFECT OF ELEVATED UV-B IRRADIATION ON THE NODULATION AND NITROGEN METABOLISM IN *VIGNA UNGUICULATA* (L.) WALP CV. COFC-8

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## ABSTRACT
Many microorganisms fix nitrogen symbiotically by partnering with a host plant. The plant provides sugars from photosynthesis that are utilized by the nitrogen-fixing microorganism for the energy it needs for nitrogen fixation. In exchange for these carbon sources, the microbe provides fixed nitrogen to the host plant for its growth. As nitrogen is the key substance apart from water that limits productivity of plants, the legume - *Rhizobium* association is vital for the effective use of atmospheric nitrogen. In recent years there is widespread concern over the global climate as the ozone layer is deteriorating due to the release of pollution containing the chemicals chlorine and bromine. Such deterioration allows large amounts of ultraviolet-B rays to reach the earth affecting the plants, microbes and their symbiotic biological nitrogen fixation. The present study is an attempt to assess the UV-B effects on nitrogen metabolism in the leaf and root system of *Vigna unguiculata* (L.) Walp cv. COFC-8. The nodulation and nitrogen metabolism on 30 DAS (days after seed germination) of *Vigna unguiculata* (L.) Walp cv. COFC-8 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. COFC-8 in the leaves by 31 and 29 % respectively, increased nitrate by 46 and 52 % and reduced nitrite by 38 and 30 % in the leaves and the root nodules. UV-B exposure enhanced NRA (nitrate reductase activity) by 38 % in leaves and by 61 % in nodules. Nodulation was suppressed by UV-B as the number of root nodules (48 %) and fresh mass of root nodules (57 %) were far below controls. UV-B stress inhibited nitrogenase enzyme activity by 28 % in roots and by 40 % in root nodules. Under ozone depleted environmental condition, an increasing UV-B radiation will dramatically affect nitrogen fixation in legumes, which in turn may have severe influence on the nitrogen budget of the earth.

***Keywords:*** *Ultra Violet-B Stress, Vigna Unguiculata, Nodules, Root Nodules, Nitrogen Metabolism*

## INTRODUCTION
Surface-level ultraviolet (UV)-B radiation (280-320 nm) is a component of the global climate and any increases in its level can lead to adverse effects on crop growth and productivity on a broad geographic scale. Possible increases in surface UV-B radiation are attributed to the depletion of the beneficial stratospheric ozone ($O_3$) layer due to the release of pollution containing the chemicals chlorine and bromine. On the other hand, 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. An increase in the flux of ultraviolet-B (UV-B) radiation is an atmospheric stress and is harmful to plant growth (Caldwell *et al.,* 1998; Rajendiran and Ramanujam, 2000; Rajendiran and Ramanujam, 2003; Rajendiran and Ramanujam, 2004) and leaf development (Kokilavani and Rajendiran, 2013). At the metabolism level, it severely inhibits photosynthesis (Caldwell *et al*., 1998; Kulandaivelu and Lingakumar, 2000; Rajendiran, 2001) and hampers nodulation and nitrogen fixation (Balakumar *et al.,* 1993; Rachel and Santhaguru, 1999; Rajendiran and Ramanujam, 2006; Sudaroli Sudha and Rajendiran, 2013a; Sudaroli and Rajendiran, 2013b; Arulmozhi and Rajendiran 2014a; Arulmozhi and Rajendiran 2014b; Vijayalakshmi and Rajendiran, 2014) in sensitive plants. Even though plants respond quickly to increases in UV-B flux by developing tolerance to some extent, the present study was carried out to analyse the

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extent of damage caused by supplementary UV-B on nodulation and nitrogen metabolism of *Vigna unguiculata* (L.) Walp cv. COFC-8, a root nodulating crop.

**MATERIALS AND METHODS**

*Vigna unguiculata* (L.) Walp cv. COFC-8 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 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 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**

Elevated UV-B radiation decreased the protein and amino acid contents of *Vigna unguiculata* (L.) Walp cv. COFC-8 in the leaves by 31 and 29 % respectively (Figure 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 nitrite in the root nodules (Figure 1). UV-B stressed plants showed reduction in nitrite by 38 and 30 % but an enhancement in nitrate content by 46 and 52 % in leaves and root nodules respectively (Figure 1). UV-B induced reduction in leaf and root nodule nitrite content was supported by Sudaroli and Rajendiran (2013a) in *Sesbania grandiflora* (L.) Pers., Sudaroli and Rajendiran (2013b) in *Vigna unguiculata* (L.) Walp. c.v. BCP-25, Arulmozhi and Rajendiran (2014a) in *Lablab purpureus* L. var. CO-5, Arulmozhi and Rajendiran (2014b) in *Lablab purpureus* L. var. Goldy and by Vijayalakshmi and Rajendiran (2014) in *Phaseolus vulgaris* L. cv. Prevail. 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 enhanced NRA by 38 % in leaves while suppressing by 26 % in root 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). 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 observation of Guerrero *et al.,* (1981) that nitrate accumulation occurred consequent to UV-B induced inhibition of NRA was confirmed by this study in the root nodules. However, Balakumar *et al.,* (1993), Sudaroli and Rajendiran (2013a),

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Sudaroli and Rajendiran (2013b), Arulmozhi and Rajendiran (2014a), Arulmozhi and Rajendiran (2014b) and Vijayalakshmi and Rajendiran (2014) did not support this view, as they have recorded suppression of both NRA and nitrate in UV-B sensitive plants as was observed with leaf under UV-B stress in the present work. Ghisi *et al.*, (2002) opined that 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 (48.65 %), their size and fresh mass of (57.89 %) were far below controls (Figure 1).

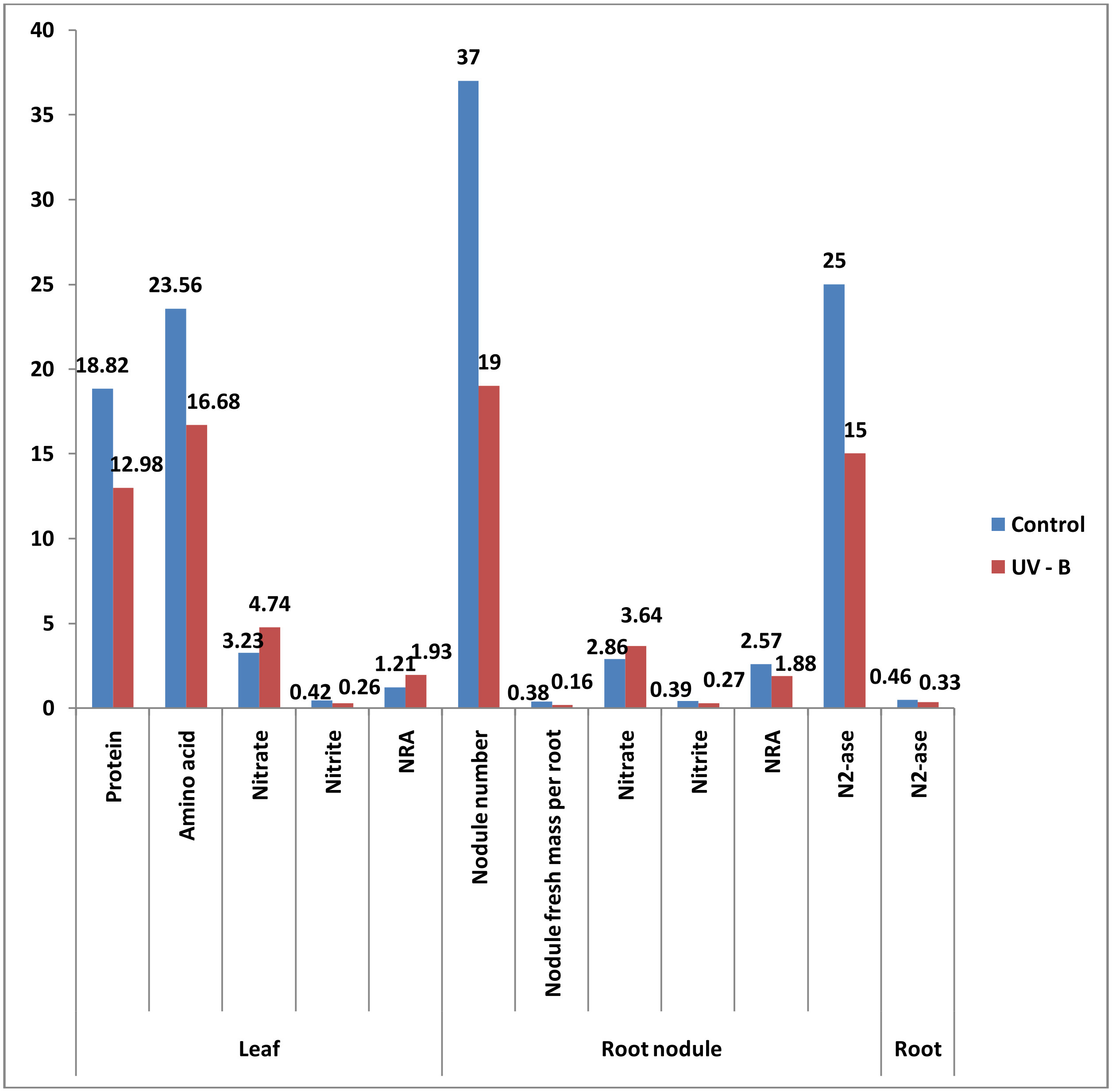


**Figure 1: Changes in number and fresh mass of nodules (g) per root system and per shoot system, contents of proteins [mg $g^{-1}$(f.m.)], amino acids, nitrates and nitrites [mg $g^{-1}$(d.m.)], and the activities of nitrate reductase, NRA [µmol($NO_2$-) $kg^{-1}$(f.m.) $s^{-1}$] and nitrogenase, $N_2$-ase [µmol(ethylene reduced) $g^{-1}$(f.m.) $s^{-1}$] in the 30 DAS leaves, roots and nodules of *Vigna unguiculata* (L.) Walp cv. COFC-8 exposed to supplementary UV-B radiation. Means followed by different letters are significantly different at $P$ = 0.05, $n$ = 10**

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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 40 % in roots and by 28.26 % in root nodules (Figure 1). Similar inhibition of nitrogenase enzyme activity after UV-B exposure was also reported by Sudaroli and Rajendiran (2013a) in *Sesbania grandiflora* (L.) Pers., Sudaroli and Rajendiran (2013b) in *Vigna unguiculata* (L.) Walp. c.v. BCP-25, Arulmozhi and Rajendiran (2014a) in *Lablab purpureus* L. var. CO-5, Arulmozhi and Rajendiran (2014b) in *Lablab purpureus* L. var. Goldy and by Vijayalakshmi and Rajendiran (2014) in *Phaseolus vulgaris* L. cv. Prevail. To conclude, UV-B stress which was proved to be detrimental to aerial parts of the plants causing photodamage of photosystem II, also disturbs the vital functions of the root system in general and the legume - Rhizobium symbiotic nitrogen fixation in particular.

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