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Research Article

IMPACT OF ULTRAVIOLET-B RADIATION OF NODULATION AND NITROGEN METABOLISM IN *VIGNA UNGUICULATA* (L.) WALP CV. COVU-1

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ABSTRACT
Nitrogen fixation is a process by which nitrogen in the atmosphere is converted into ammonia. Atmospheric nitrogen or molecular nitrogen is relatively inert and it does not easily react with other chemicals to form new compounds. Fixation processes free up the nitrogen atoms from their diatomic form to be used in other ways. Symbiotic nitrogen fixation is an important process that occurs in legumes which harbour nitrogen-fixing bacteria within their tissues. In recent years, human activities have destroyed the protective ozone layer allowing enormous amount of ultraviolet-B (UV-B) radiation into earth's surface. The present work was designed for assessing the potential impact of enhanced levels of UV-B on the nitrogen metabolism in the leaves and roots of *Vigna unguiculata* (L.) Walp cv. COVU-1. The nodulation and nitrogen metabolism on 30 DAS (days after seed germination) of *Vigna unguiculata* (L.) Walp cv. COVU-1 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 protein and amino acid contents of *Vigna unguiculata* (L.) Walp cv. COVU-1 in the leaves by 33 and 24 % respectively, increased nitrate by 34 and 35 % and reduced nitrite by 39 % and 21 % in the leaves and the root nodules respectively. UV-B exposure enhanced NRA (nitrate reductase activity) by 60 % in leaves suppressing the activity by 24 % in nodules. Nodulation was suppressed by UV-B as the number of root nodules (47 %) and fresh mass of root nodules (30 %) were far below controls. UV-B stress also inhibited nitrogenase enzyme activity by 25 % in roots and by 68 % in root nodules. These findings suggest that drastic reductions in grain nutrient content may result with further increase in ozone depletion, as the symbiotic nitrogen fixation in the legume was heavily suppressed after exposure to elevated UV-B radiation.

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

INTRODUCTION
Stratospheric ozone depletion has caused an increase in the amount of ultraviolet-B (UV-B) radiation reaching the earth's surface. 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 Sudha and Rajendiran, 2013b; Sudaroli Sudha and Rajendiran, 2013c; Sudaroli and Rajendiran, 2013d; Sudaroli and Rajendiran, 2014; Arulmozhi and Rajendiran, 2014; Vijayalakshmi and Rajendiran, 2014) in sensitive plants.

Even though numerous investigations were carried out on the effect of UV-B radiation on plant growth, yield, photosynthesis, susceptibility to disease, changes in plant structure and pigmentation, very little information is available on its effect on nitrogen metabolism.

The present work is aimed at to record the impact of supplementary UV-B radiation on nodulation and nitrogen metabolism of *Vigna unguiculata* (L.) Walp cv. COVU-1, a root nodulating crop.

MATERIALS AND METHODS

The seeds of *Vigna unguiculata* (L.) Walp cv. COVU-1 obtained from Tamil Nadu Agriculture University, were sown in pots and were grown 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 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).

RESULTS AND DISCUSSION

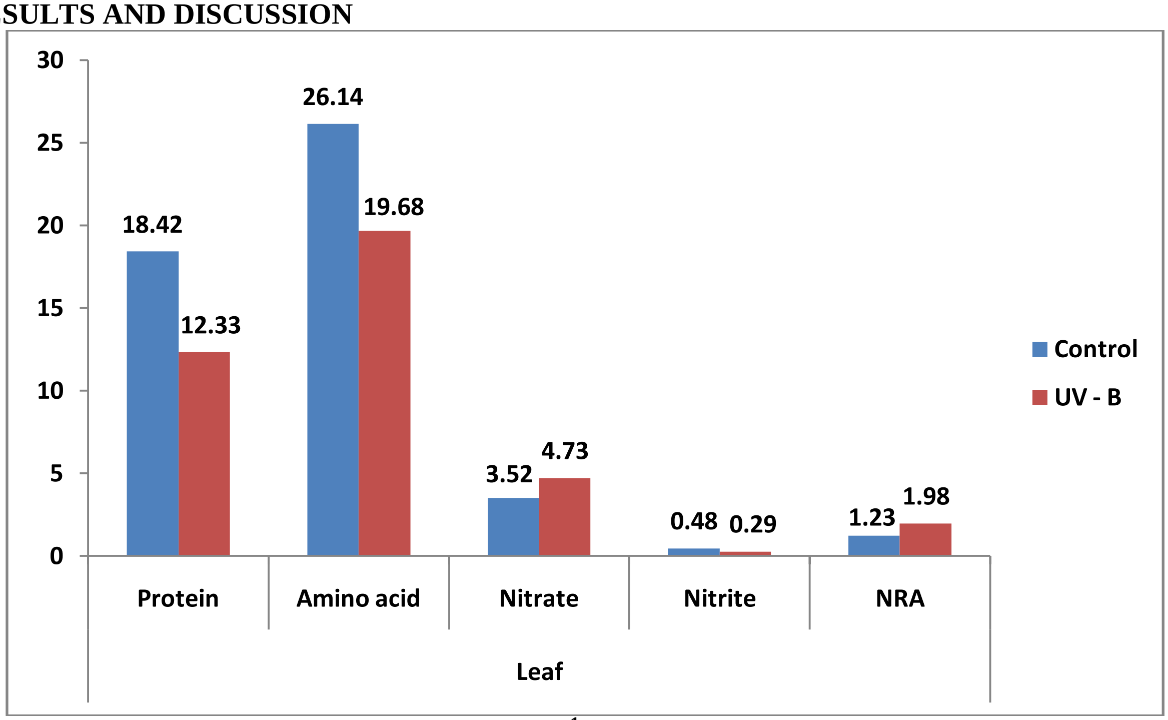

Figure 1: Changes contents of proteins [mg g^{-1}(f.m.)], amino acids, nitrates and nitrites [mg g^{-1}(d.m.)], and the activities of nitrate reductase, NRA [µmol(NO_2-) kg^{-1}(f.m.) s^{-1}] in the 30 DAS leaves of *Vigna unguiculata* (L.) Walp cv. COVU-1 exposed to supplementary UV-B radiation. Means followed by different letters are significantly different at *P* = 0.05, *n* = 10

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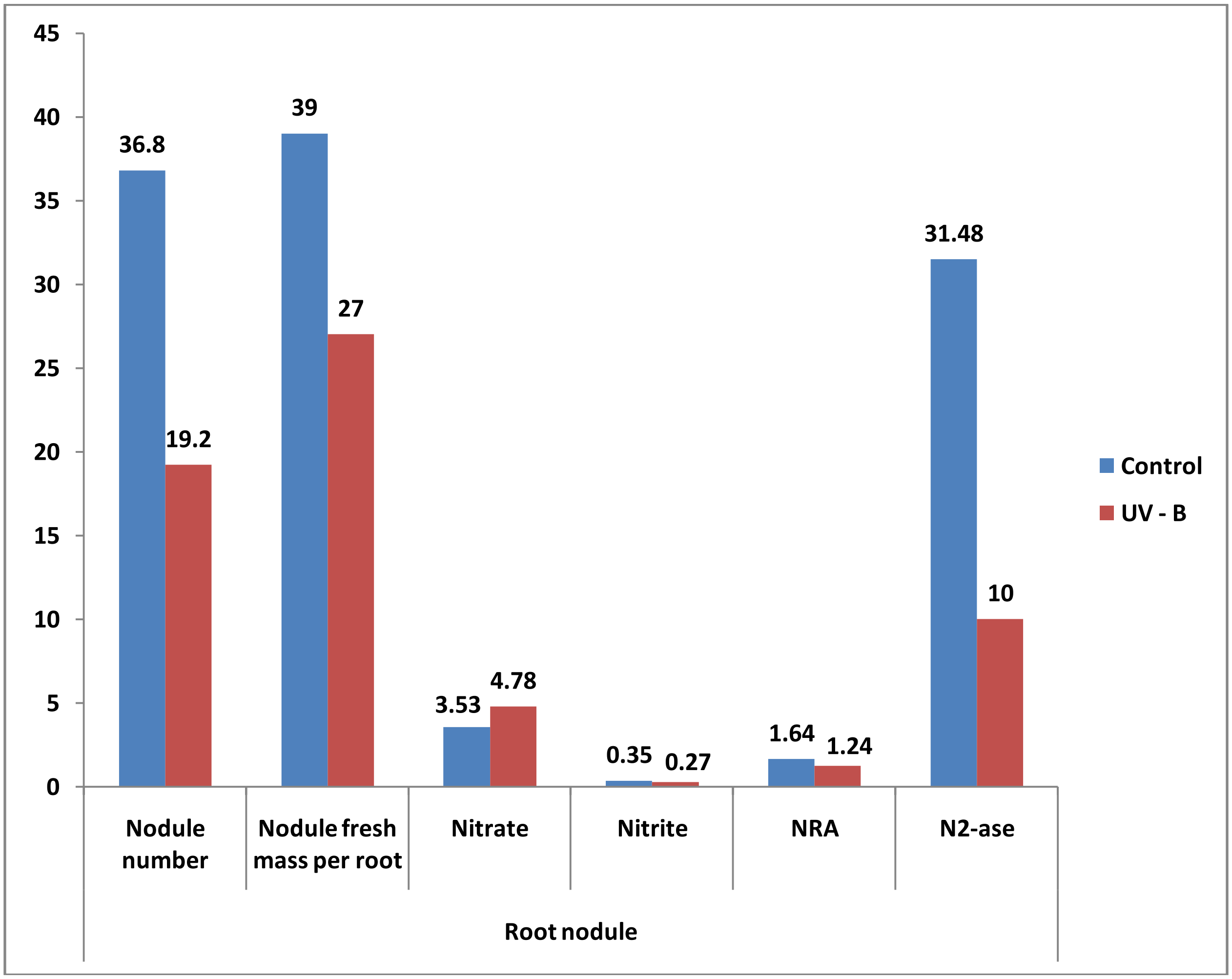

Figure 2: Changes in number and fresh mass of nodules (g) per root system, contents of nitrates and nitrites [mg g^{-1}(d.m.)], and the activities of nitrate reductase, NRA [µmol(NO_2) kg^{-1}(f.m.) s^{-1}] in the 30 DAS root nodules of *Vigna unguiculata* (L.) Walp cv. COVU-1 exposed to supplementary UV-B radiation. Means followed by different letters are significantly different at *P* = 0.05, *n* = 10

Elevated UV-B radiation decreased the protein and amino acid contents of *Vigna unguiculata* (L.) Walp cv. COVU-1 in the leaves by 33 and 24 % 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 39 and 22 % but an enhancement in nitrate content by 34 and 35 % in leaves and root nodules respectively (Figure 1, 2). Reduction in nitrite in leaf and root nodule after UV-B exposure was supported by Rajendiran and Ramanujam (2006) in *Vigna radiata* (L.) Wilczek var. KM-2, Sudaroli and Rajendiran (2013a) in *Sesbania grandiflora* (L.) Pers., Sudaroli and Rajendiran (2013b) in *Vigna unguiculata* (L.) Walp. c.v. BCP-25, Sudaroli and Rajendiran (2013c) in *Sesbania rostrata* Bremek. & Oberm., Sudaroli and Rajendiran (2013d) in black gram, Sudaroli and Rajendiran (2014) in *Sesbania aculeata* (Willd.) Pers., Arulmozhi and Rajendiran (2014) 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.

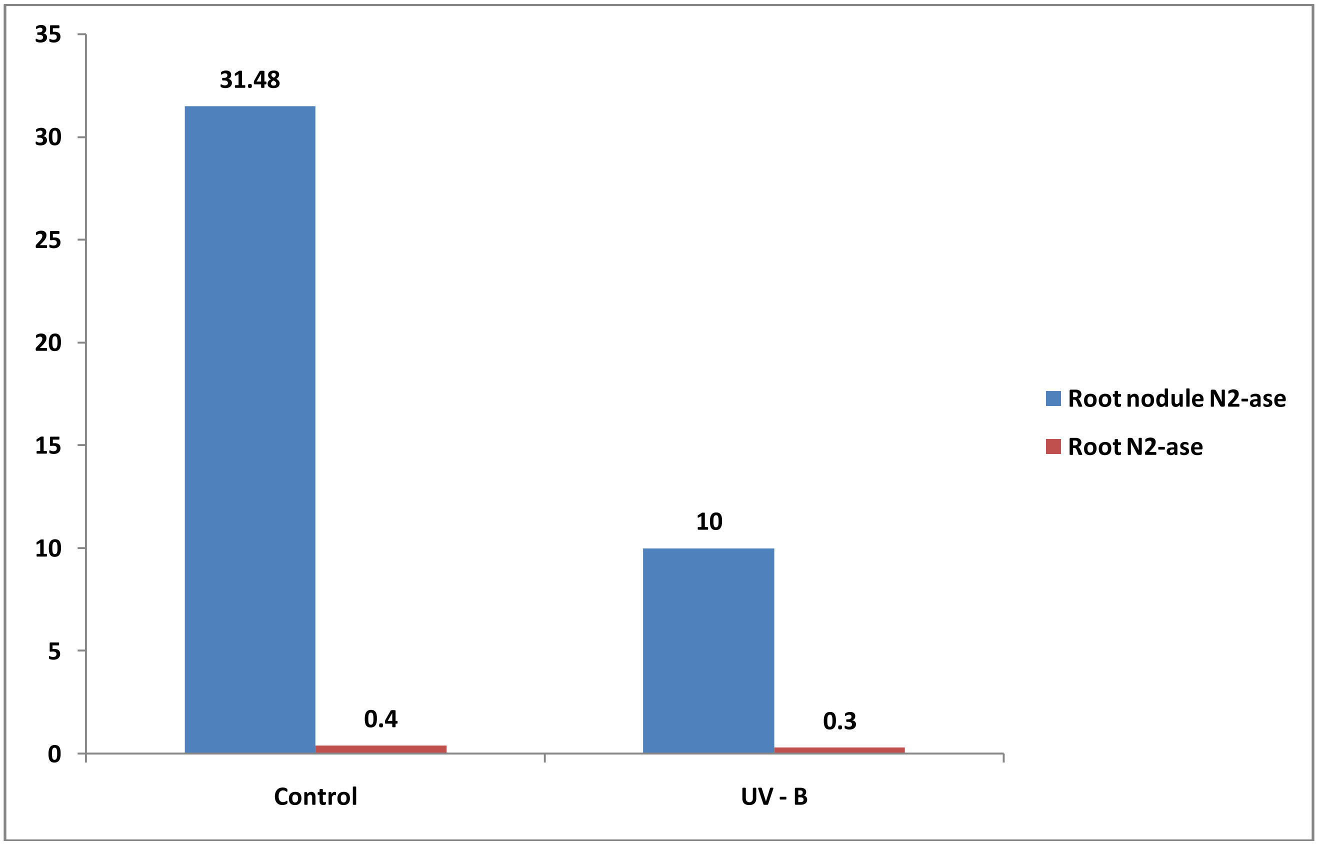

Figure 3: Changes in N_2-ase [µmol(ethylene reduced) g^{-1}(f.m.) s^{-1}] in the 30 DAS root nodules and roots of *Vigna unguiculata* (L.) Walp cv. COVU-1 exposed to supplementary UV-B radiation. Means followed by different letters are significantly different at *P* = 0.05, *n* = 10

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 60 % 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). 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), Sudaroli and Rajendiran (2013b), Sudaroli and Rajendiran (2013c), Sudaroli and Rajendiran (2013d), Sudaroli and Rajendiran (2014), Arulmozhi and Rajendiran (2014) and Vijayalakshmi and Rajendiran (2014) did not support this view, as they have recorded suppression of both NRA and nitrate in UV-B exposed plants. 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 (47 %), their size and fresh mass of (30 %) were far below controls (Figure 2). 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 24 % in roots and by 68 % in root nodules (Figure 3).

Similar inhibition of nitrogenase enzyme activity after UV-B exposure was also reported by Rajendiran and Ramanujam (2006) in *Vigna radiata* (L.) Wilczek var. KM-2, Sudaroli and Rajendiran (2013a) in *Sesbania grandiflora* (L.) Pers., Sudaroli and Rajendiran (2013b) in *Vigna unguiculata* (L.) Walp. c.v. BCP-25, Sudaroli and Rajendiran (2013c) in *Sesbania rostrata* Bremek. & Oberm., Sudaroli and Rajendiran (2013d) in black gram, Sudaroli and Rajendiran (2014) in *Sesbania aculeata* (Willd.) Pers., Arulmozhi and Rajendiran (2014) in *Lablab purpureus* L. var. Goldy and by Vijayalakshmi and Rajendiran (2014) in *Phaseolus vulgaris* L. cv. Prevail. UV-B stress which was earlier proved to be detrimental to aerial parts of the plants also disturbs the legume-Rhizobium symbiotic nitrogen fixation in the root system of *Vigna unguiculata* (L.) Walp cv. COVU-1.

Conclusion

To conclude, further destruction of the naturally occurring ozone layer might enhance UV-B stress on the symbiotic nitrogen fixation in legumes, thereby reducing the nutrient content of the grains.

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