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Research Article

EFFECT OF ELEVATED UV-B IRRADIATION ON THE NODULATION AND NITROGEN METABOLISM IN *VIGNA UNGUICULATA* (L.) WALP. CV. CW-122

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ABSTRACT
Nitrogen is a critical chemical element in both proteins and nucleic acids, and thus every living organism must metabolize nitrogen to survive. The legume - *Rhizobium* symbiotic relationship provides a major route for harnessing the gaseous nitrogen from the atmosphere. Pollution created by human activites directly depletes the naturally formed ozone layer by chemicals like chlorine and bromine and indirectly by global warming. In recent years depletion in ozone layer allows enormous ultraviolet-B (UV-B) radiation into earth's surface affecting plant growth. The present study is an attempt to assess the extent of damage caused by UV-B on the nitrogen metabolism of *Vigna unguiculata* (L.) Walp. cv. CW-122. The nodulation and nitrogen metabolism on 30 DAS (days after seed germination) of *Vigna unguiculata* (L.) Walp. cv. CW-122 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. CW-122 in the leaves by 37 and 29.17 % respectively, accumulated nitrate by 56 and 33% and suppressed nitrite by 41.02 % and 25 % in in the leaves and root nodules respectively. UV-B exposure suppressed NRA (nitrate reductase activity) by 51.32 % in leaves and 23.53 % in root nodules. Nodulation was suppressed by UV-B as the number (44.22 %) and fresh mass of root nodules (38 %) were reduced far below controls. In addition UV-B rays also inhibited nitrogenase enzyme activity by 29.55 % in roots and by 63.12 % in root nodules. Present study proves that enhanced UV-B irradiation plays a direct role in suppressing the symbiotic nitrogen fixation in legumes.

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

INTRODUCTION
An increase in the flux of ultraviolet-B (UV-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) and leaf architecture (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; Arulmozhi and Rajendiran, 2014; Vijayalakshmi and Rajendiran, 2014; Vijayalakshmi *et al.*, 2014) in sensitive plants. The present study was to assess the influence of increased UV-B flux on nodulation and nitrogen metabolism of *Vigna unguiculata* (L.) Walp. cv. CW-122, a root nodulating plant and an important member of symbiotic nitrogen fixation in agriculture.

MATERIALS AND METHODS
Vigna unguiculata (L.) Walp. cv. CW-122 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-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. CW-122 in the leaves by 37 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). However UV-B stressed plants showed enhanced nitrate by 56 and 33 % in the leaves and nodules and reduced nitrite by 41.02 and 25 % in the leaf and the root nodules respectively (Figure 1). This is in accordance with the report of Sudaroli Sudha and Rajendiran (2013a) in *Sesbania grandiflora* (L.) Pers., Sudaroli Sudha and Rajendiran (2013b) in *Vigna unguiculata* (L.) Walp. c.v. BCP-25, Arulmozhi and Rajendiran (2014) in *Lablab purpureus* L. var. Goldy, Vijayalakshmi and Rajendiran (2014) in *Phaseolus vulgaris* L. cv. Prevail and Vijayalakshmi *et al.,* (2014) in *Cyamopsis tetragonoloba* (L) TAUB. var. PNB. In barley (Ghisi *et al.*, 2002) and in *Vigna radiata* (Rajendiran and Ramanujam, 2006) there were 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 suppressed NRA by 51 % in leaves and 23 % 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 nitrate accumulation consequent to suppression of NRA under UV-B stress as observed by Guerrero *et al.*, (1981) was confirmed by this study. However, Balakumar *et al.*, (1993), Sudaroli Sudha and Rajendiran (2013a), Sudaroli Sudha and Rajendiran (2013b), Arulmozhi and Rajendiran (2014), Vijayalakshmi and Rajendiran (2014) and Vijayalakshmi *et al.*, (2014) did not support this view, as they have recorded suppression of both NRA and nitrate in leaves and root nodules of UV-B stressed crops. 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 (44 %), size and fresh mass of root nodules (38 %) were far below controls (Figure 1). 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). Sudaroli Sudha and Rajendiran (2013a) in *Sesbania grandiflora* (L.) Pers., Sudaroli Sudha and Rajendiran. (2013b) in *Vigna unguiculata* (L.) Walp. c.v. BCP-25, Arulmozhi and Rajendiran (2014) in *Lablab purpureus* L. var. Goldy, Vijayalakshmi and Rajendiran (2014) in *Phaseolus vulgaris* L. cv. Prevail and Vijayalakshmi *et al.*, (2014) in *Cyamopsis tetragonoloba*

(L) TAUB. var. PNB have reported reductions in nitrogenase enzyme activity subsequent to UV-B exposure. The present study supported the earlier reports, as the UV-B stressed legumes exhibited reduction in nitrogenase enzyme activity by 29 % in roots and by 63 % in root nodules (Figure 1).

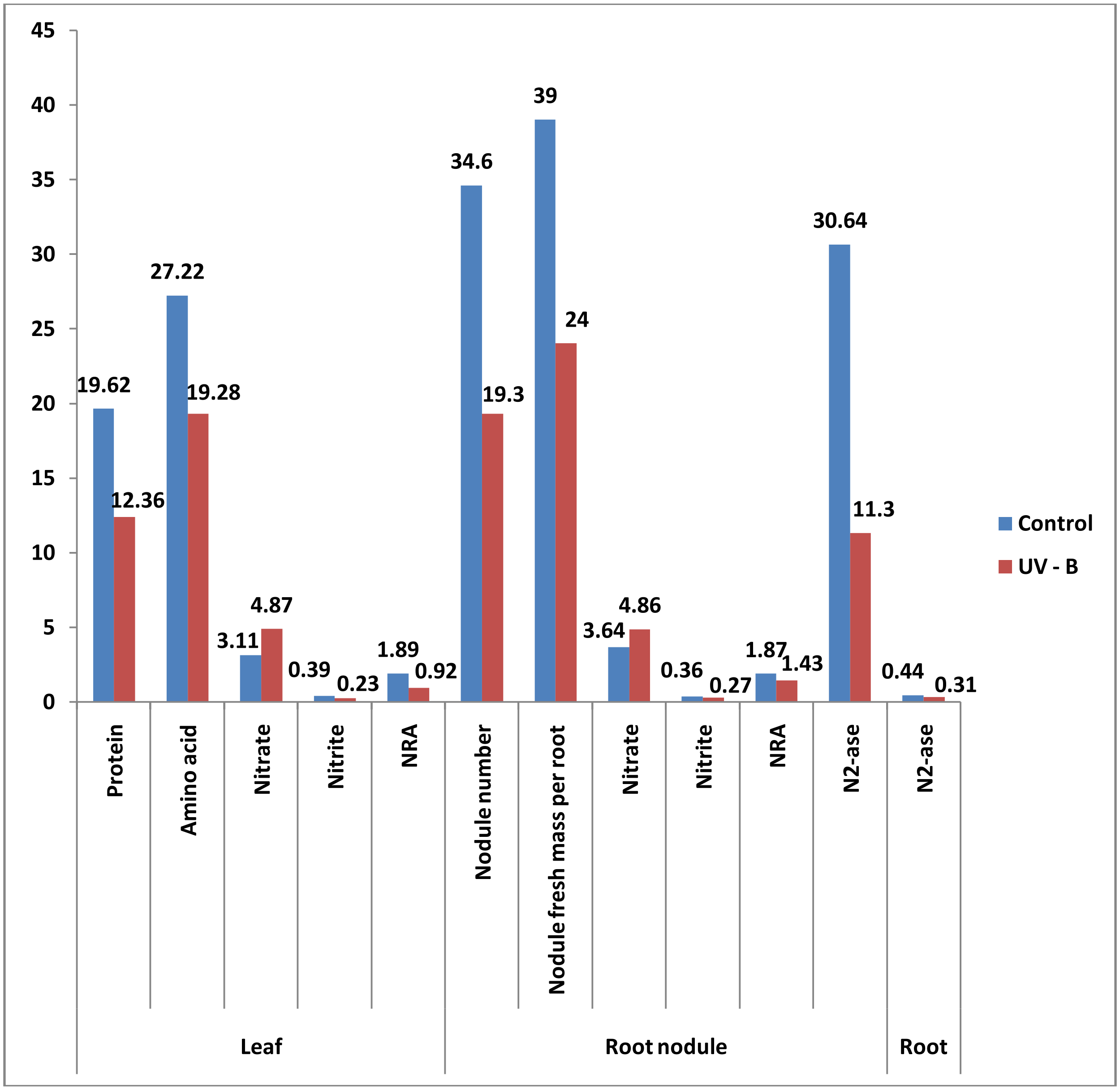

Figure 1: Changes in number and fresh mass of nodules (g) per root system and per shoot system, contents of proteins [mg g^{-1}(f.m.)], amino acids, nitrates and nitrites [mg g^{-1}(d.m.)], and the activities of nitrate reductase, NRA [µmol(NO_2-) kg^{-1}(f.m.) s^{-1}] and nitrogenase, N_2-ase [µmol(ethylene reduced) g^{-1}(f.m.) s^{-1}] in the 30 DAS leaves, roots and nodules of *Vigna unguiculata* (L.) Walp. cv. CW-122 exposed to supplementary UV-B radiation. Means followed by different letters are significantly different at *P* = 0.05, *n* = 10

Hence, UV-B stress which was known to impair the photosynthetic apparatus of the leaves of the crops also denatures the enzymes required to carry out symbiotic nitrogen fixation in the root nodules of legumes.

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