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Research Article

ULTRA VIOLET-B INDUCED REDUCTION IN NODULATION AND NITROGEN METABOLISM IN *VIGNA UNGUICULATA* (L.) WALP CV. CO-1

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ABSTRACT

The nitrogen fixed by a legume in any environment is regulated by the amount of nitrogen present in the legume's products of growth and the proportion of nitrogen derived from atmospheric nitrogen as a result of symbiotic nitrogen fixation by *Rhizobia* in the root nodules. In recent years, depletion of ozone gas in the useful stratospheric ozone layer allows ultraviolet-B (UV-B) radiation into earth's surface affecting the growth of crops and disturbing various metabolisms in the aerial parts. The present study is an attempt to assess the UV-B effects on the symbiotic nitrogen fixation in *Vigna unguiculata* (L.) Walp cv. CO-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}), the nodulation and nitrogen metabolism on 30 DAS (days after seed germination) of *Vigna unguiculata* (L.) Walp cv. CO-1 was monitored. UV-B irradiation decreased the protein and amino acid contents of *Vigna unguiculata* (L.) Walp cv. CO-1 in the leaves by 35.91 and 30.31 % respectively, increased nitrate by 29.53 and 28.31 % and reduced nitrite by 46.15 % and 28.95 % in the leaves and the root nodules respectively. UV-B exposure suppressed NRA (nitrate reductase activity) by 43.64 % in leaves and by 20.97 % in nodules. Nodulation was suppressed by UV-B as the number of root nodules (39.90 %) and fresh mass of root nodules (41.77 %) were far below controls. UV-B stress also inhibited nitrogenase enzyme activity by 30.95% in roots and by 61.44 % in root nodules. Present study proves that enhancement of UV-B stress on legumes affects the symbiotic nitrogen fixation in the root nodules.

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

INTRODUCTION

Rhizobium is the most well known species of a group of bacteria that acts as the primary symbiotic fixer of nitrogen. These bacteria can infect the roots of leguminous plants, leading to the formation of lumps or nodules where the nitrogen fixation takes place. The bacterium's enzyme system supplies a constant source of reduced nitrogen to the host plant and the plant furnishes nutrients and energy for the activities of the bacterium. 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; Kokilavani and Rajendiran, 2014a; Kokilavani and Rajendiran, 2014b). 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 and Rajendiran, 2013a; Sudaroli and Rajendiran, 2013b; Sudaroli and Rajendiran, 2013c; Sudaroli and Rajendiran, 2013d; Sudaroli and Rajendiran 2014a; Sudaroli and Rajendiran, 2014b; Arulmozhi and Rajendiran, 2014a; Arulmozhi and Rajendiran, 2014b; Arulmozhi and Rajendiran, 2014c; Vijayalakshmi and Rajendiran, 2014a; Vijayalakshmi and Rajendiran, 2014b; Vijayalakshmi and Rajendiran, 2014c) in sensitive plants. The present work was carried out to estimate the damage caused by elevated UV-B rays on nodulation and nitrogen fixation in *Vigna unguiculata* (L.) Walp cv. CO-1.

MATERIALS AND METHODS

Vigna unguiculata (L.) Walp cv. CO-1 seeds obtained from Tamil Nadu Agricultural University, Coimbatore, 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) and this dosage was maintained by adjusting the height of the lamps over the canopy. 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

Supplementary UV-B irradiation which affected the aerial parts of the *Vigna unguiculata* (L.) Walp cv. CO-1 crops continued to suppress the root system, as the roots were weak with severely reduced nodules (Plate 1: Figure 1 to 4). The nodules under UV-B stress were under developed due to reduced inoculum by *Rhizobium* as against the well developed nodules with rich *Rhizobium* in normal plants (Plate 1: Figure 5 to 8). The protein and amino acid contents of leaves in *Vigna unguiculata* (L.) Walp cv. CO-1 decreased by 35.91 and 30.31 % respectively (Plate 2). 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 (Plate 3). UV-B stressed plants showed reduction in nitrite by 46.15 and 28.95 % but an enhancement in nitrate content by 29.53 and 28.31 % in leaves and root nodules respectively (Plate 2, 3). 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 Sudha and Rajendiran (2013a) in *Sesbania grandiflora* (L.) Pers., Sudaroli and Rajendiran (2013b) in *Vigna unguiculata* (L.) Walp. cv. BCP-25, Sudaroli and Rajendiran (2013c) in *Sesbania rostrata* Bremek. & Oberm., Sudaroli and Rajendiran (2013d) in black gram, Sudaroli and Rajendiran (2014a) in *Sesbania aculeata* (Willd.) Pers., Sudaroli and Rajendiran (2014b) in *Vigna unguiculata* (L.) Walp. cv. COVU-1, Arulmozhi and Rajendiran (2014a) in *Lablab purpureus* L. var. Goldy, Arulmozhi and Rajendiran (2014b) in hyacinth bean, Arulmozhi and Rajendiran (2014c) in *Vigna unguiculata* (L.) Walp. cv. COFC-8, Vijayalakshmi and Rajendiran (2014a) in *Phaseolus vulgaris* L. cv. Prevail, Vijayalakshmi and Rajendiran (2014b) in *Cyamopsis tetragonoloba* (L.) Taub. var. PNB and by Vijayalakshmi and Rajendiran (2014c) in *Vigna unguiculata* (L.) Walp. cv. CW-122. 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 suppressed NRA by 43.64 % in leaves and by 20.97 % in root nodules (Plate 2, 3). 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) and in *Vigna radiata* (L.) Wilczek var. KM-2 (Rajendiran and Ramanujam 2006). 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), Arulmozhi and Rajendiran (2014c) and Vijayalakshmi and Rajendiran (2014c) that nitrate accumulation occurred consequent to UV-B induced inhibition of NRA was confirmed by this study (Plate 2, 3). However Balakumar *et al.*, (1993), Rajendiran and Ramanujam (2006), Sudaroli and Rajendiran (2013a), Sudaroli and Rajendiran (2013b), Sudaroli and Rajendiran (2013c), Sudaroli and Rajendiran (2013d), Sudaroli and Rajendiran (2014a), Sudaroli and Rajendiran (2014b), Arulmozhi and Rajendiran (2014a), Arulmozhi and Rajendiran (2014b), Vijayalakshmi and Rajendiran (2014a) and Vijayalakshmi and Rajendiran (2014b) 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 (39.90 %), their size and fresh mass of (41.77 %) were far below controls (Plate 3).

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 30.95 % in roots and by 61.44 % in root nodules (Plate 3, 4). 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 (2014a) in *Sesbania aculeata* (Willd.) Pers., Arulmozhi and Rajendiran (2014a) in *Lablab purpureus* L. var. Goldy and by Vijayalakshmi and Rajendiran (2014a) in *Phaseolus vulgaris* L. cv. Prevail. From the depressed nitrogen metabolism recorded in *Vigna unguiculata* (L.) Walp cv. CO-1, it is evident that ultraviolet-B radiation is a dangerous environmental stress which can adversely affect the nitrogen fixing ability of the legumes.

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PLATE 1

Comparison of normal and supplementary UV-B irradiated 30 days old plants, leaves, roots and root nodules (entire and cross section) of *Vigna unguiculata* (L.) Walp cv. CO-1.

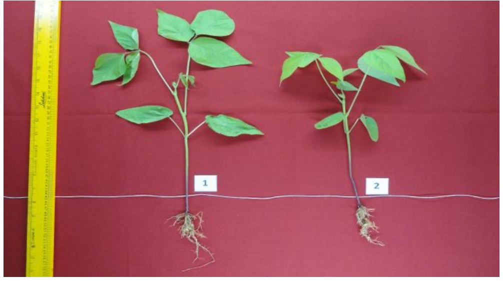

Fig. 1: Control and UV-B stressed plants

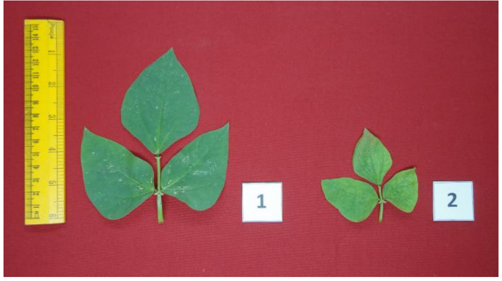

Fig. 2: Control and UV-B stressed leaves

Fig. 3: Control and UV-B stressed roots

Fig. 4: Control and UV-B stressed nodules

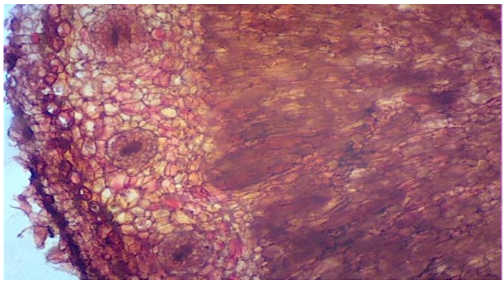

Fig. 5: Control – Well developed nodule

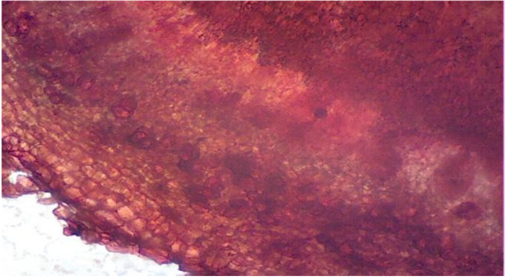

Fig. 6: UV-B – Under developed nodule

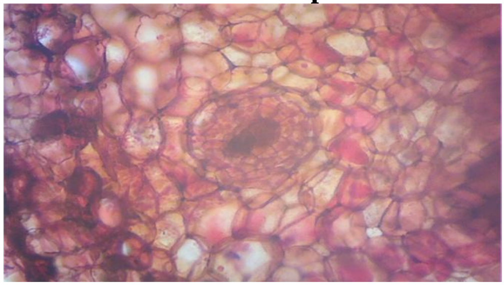

Fig. 7: Control – *Rhizobium* zone

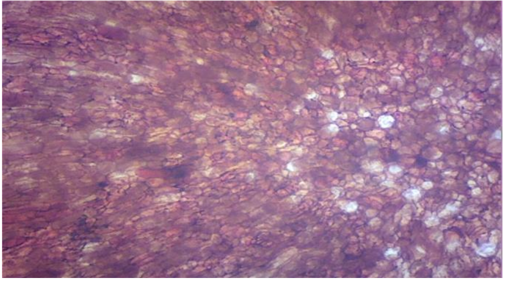

Fig. 8: UV-B – Devoid of *Rhizobium*

PLATE 2

Changes in contents of proteins [mg g^{-1}(f.m.)], amino acids, nitrates and nitrites [mg g^{-1} (d.m.)], and the activity of nitrate reductase, NRA [µmol (NO_2-) kg^{-1} (f.m.) s^{-1}] in the 30 DAS leaves of *Vigna unguiculata* (L.) Walp cv. CO-1 exposed to supplementary UV-B radiation.

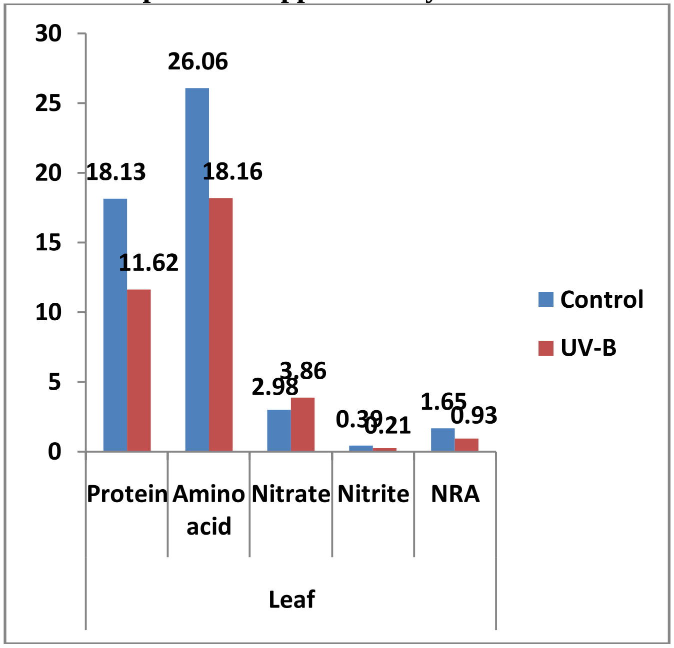

PLATE 3

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}] and nitrogenase, N_2-ase [µmol (ethylene reduced) g^{-1} (f.m.) s^{-1}] in the 30 DAS root nodules of *Vigna unguiculata* (L.) Walp cv. CO-1 exposed to supplementary UV-B radiation.

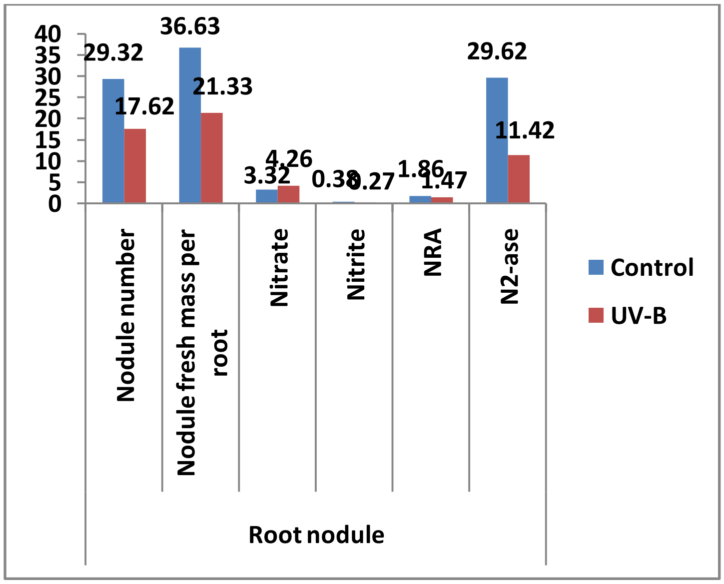

PLATE 4

Changes in the activity of N_2-ase [µmol (ethylene reduced) g^{-1} (f.m.) s^{-1}] in the 30 DAS roots of *Vigna unguiculata* (L.) Walp cv. CO-1 exposed to supplementary UV-B radiation.