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ULTRA VIOLET-B INDUCED REDUCTION IN NODULATION AND NITROGEN METABOLISM IN *VIGNA MUNGO* L. VAR. T-9 BY ELEVATED UV-B RADIATION

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ABSTRACT
Nitrogen fixation by *Rhizobia* is of great importance in agriculture as the legumes feed for the meat-producing animals of the world as well as humans. Crop yields are greatly improved in nodulated plants as the legumes can grow well even in poor soils with very little fixed nitrogen. After harvest legume roots left in the soil decay, releasing organic nitrogen compounds for uptake by the next generation of plants through crop rotation. Stratospheric ozone filters out most of the sun's potentially harmful shortwave ultraviolet (UV) radiation. This ozone has become depleted, due to the release of such ozone-depleting substances as chlorofluorocarbons (CFCs). When stratospheric ozone is depleted, more UV rays reach the earth. Exposure to higher amounts of UV radiation could have serious impacts on human beings, animals and plant. The present study was carried out to examine the ultraviolet-B (UV-B) radiation induced changes in symbiotic nitrogen fixation of *Vigna mungo* L. var. KM-2. 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 15, 30 and 45 DAS (days after seed germination) of *Vigna mungo* L. var. T-9 were monitored. UV-B stress decreased the protein and amino acid contents in the leaves by 24 to 33 % and 19 to 27 % respectively and reduced nitrate and nitrite by 16 to 21 % and 31 to 43% in the leaves and by 14 to 19% and 20 to 25 % in the root nodules respectively. NRA (nitrate reductase activity) was suppressed by 30 to 39 % in leaves and 18 to 23 % in nodules after UV-B exposure. The nodulation was also reduced after UV-B irradiation as the number (22 to 38 %) and fresh mass (28 to 37 %) of root nodules were far below controls. Nitrogenase enzyme activity was inhibited by 21 to 30 % in roots and by 33 to 52 % in root nodules due to UV-B radiation. Present study proves that enhanced UV-B stress on legumes depresses the symbiotic nitrogen fixation in the root nodules.

KEY WORDS: Ultraviolet-B stress, black gram, root nodules, nitrate reductase, nitrogenase, nitrogen metabolism.

INTRODUCTION
Nitrogen fixation by natural means cuts down on the use of artificial fertilizers. This not only saves money but also helps to prevent the pollution brought about by excessive use of commercial nitrogen and ammonia fertilizers such as eutrophication of rivers and lakes, generation of acid rain, and overgrowth of agricultural land by non-food crops. 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 and 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 Sudha and Rajendiran 2013d, Sudaroli Sudha and Rajendiran 2014, Arulmozhi and Rajendiran, 2014 and Vijayalakshmi and Rajendiran, 2014) in sensitive plants. The present study is an attempt to assess the influence of supplementary UV-B radiation on nodulation and nitrogen metabolism in yet another root nodulating legume, *Vigna mungo* L. var. T-9.

MATERIALS & METHODS
Vigna mungo L. var. T-9 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 ± 2ºC, 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 15, 30 and

45 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 & DISCUSSION

The protein and amino acid contents of leaves in *Vigna mungo* L. var. T-9 were decreased by 24 to 33% and 19 to 27% respectively after exposure to supplementary UV-B radiation (Figure 1).

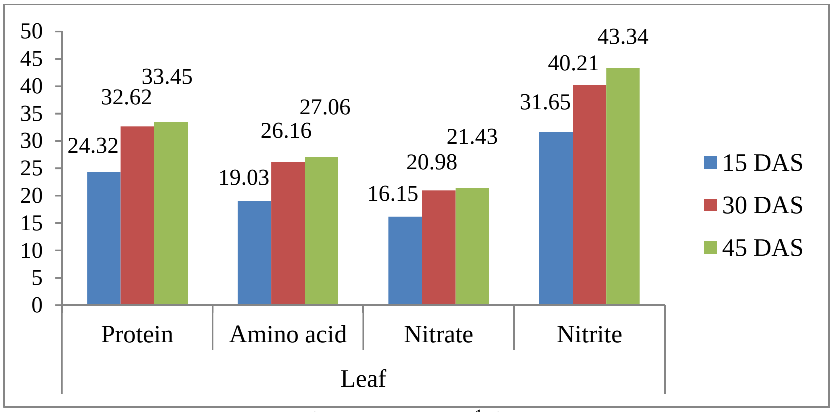

FIGURE 1: Percentage reduction in the contents of proteins [mg g^{-1}(f.m.)], amino acids, nitrates and nitrites [mg g^{-1}(d.m.)] in the 15, 30 and 45 DAS leaves of *Vigna mungo* L. var. T-9 exposed to supplementary UV-B radiation

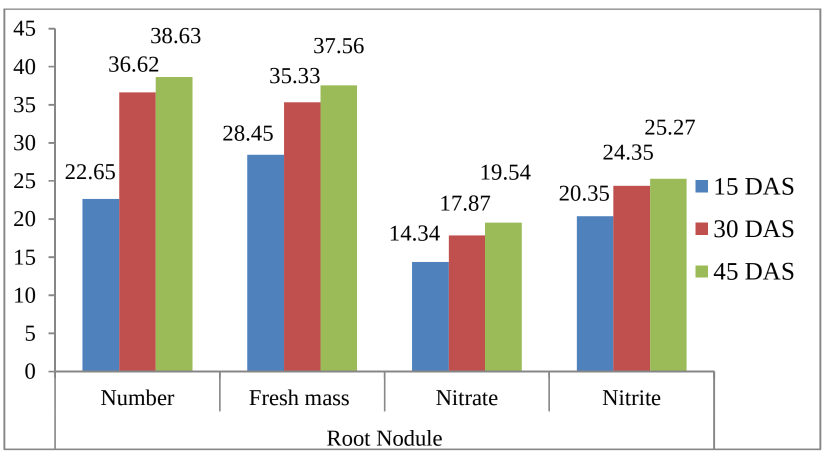

FIGURE 2: Percentage reduction in number and fresh mass of nodules (g) per root system, nitrates and nitrites [mg g^{-1}(d.m.)] in the 15, 30 and 45 DAS root nodules of *Vigna mungo* L. var. T-9 exposed to supplementary UV-B radiation.

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, Sudha and Rajendiran, 2013a, Sudha and Rajendiran, 2013b). In contrast to unstressed plants accumulating more nitrate and nitrite in the root nodules, UV-B irradiated plants showed reduction by 16 to 21% and 31 to 43% in the leaves and by 14 to 19% and 20 to 25% in the root nodules respectively (Figure 1, 2). Reduction in nitrite and nitrite in the leaves as well as root nodules after UV-B exposure was in accordance with Rajendiran and Ramanujam (2006) in *Vigna radiata* (L.) Wilczek var. KM-2, Sudaroli Sudha and Rajendiran (2013a) in *Sesbania grandiflora* (L.) Pers., Sudaroli Sudha and Rajendiran. (2013b) in *Vigna unguiculata* (L.) Walp. c.v. BCP-25, Sudaroli Sudha and Rajendiran (2013c) in *Sesbania rostrata* Bremek. & Oberm., Sudaroli Sudha and Rajendiran (2014) in *Sesbania aculeata* (Willd.) Pers., Arulmozhi and Rajendiran (2014) in *Lablab purpureus* L. var. Goldy and Vijayalakshmi and Rajendiran (2014) in *Phaseolus vulgaris* L. cv. Prevail. UV-B exposure suppressed NRA by 30 to 39% in leaves and 18 to 23% in nodules (Figure 3). Similar results of decreased values of NRA after exposure to UV-B radiation in comparison with control seedlings were reported by Quaggiotti *et al.* (2004) in the leaves and roots of *Zea mays* L. and in *Vigna radiata* (L.)

Wilczek var. KM-2, Rajendiran and Ramanujam (2006). Significant reductions in the activities of nitrate reductase were observed by Ghisi *et al.* (2002) in barley, Rajendiran and Ramanujam (2006) in *Vigna radiata*, Sudha and Rajendiran (2013a) in *Sesbania grandiflora,* Sudha and Rajendiran (2013b) in *Sesbania rostrata,* Arulmozhi and Rajendiran (2014) in *Lablab purpureus* L. var. Goldy and Vijayalakshmi and Rajendiran (2014) in *Phaseolus vulgaris* L. cv. Prevail, not only in the UV-B receiving leaves but also in the root nodules. Chimphango *et al*. (2003) reported no adverse effect of elevated UV-B radiation on growth and symbiotic function of *Lupinus luteus* and *Vicia atropurpurea* plants. The 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, Rajendiran, 2001). However an enhancement of NRA after exposure to UV-B irradiance was reported by Marek, *et al.* (2008) in *Pinus sylvestris* L. needle. The nitrate accumulation consequent to UV-B induced inhibition of NRA was observed by Guerrero *et al*. (1981) but was not confirmed by this study. Such a disparity was reported in UV-B irradiated *Vigna unguiculata* (Balakumar *et al*. 1993), *Vigna radiata* (L.) Wilczek var. KM-2 (Rajendiran and Ramanujam 2006), *Sesbania grandiflora* (L.) Pers. (Sudaroli Sudha and Rajendiran 2013a), *Vigna unguiculata* (L.) Walp. c.v. BCP-25 (Sudaroli Sudha and Rajendiran. 2013b), *Sesbania rostrata* Bremek. & Oberm. (Sudaroli Sudha and Rajendiran, 2013c), *Sesbania aculeata* (Willd.) Pers. (Sudaroli Sudha and Rajendiran, 2014), *Lablab purpureus* L. var. Goldy (Arulmozhi and Rajendiran, 2014) and *Phaseolus vulgaris* L. cv. Prevail (Vijayalakshmi and Rajendiran, 2014). 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 root nodules (22 to 38%), size and fresh mass of root nodules (28 to 37 %) were far below controls (Figure 2). In contrast to the present study, Samson *et al.* (2004) reported that 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. Elevated UV-B stress inhibited nitrogenase enzyme activity by 21 to 30 % in roots and by 33 to 52 % in root nodules respectively (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 Sudha and Rajendiran (2013a) in *Sesbania grandiflora* (L.) Pers., Sudaroli Sudha and Rajendiran (2013b) in *Vigna unguiculata* (L.) Walp. c.v. BCP-25, Sudaroli Sudha and Rajendiran (2013c) in *Sesbania rostrata* Bremek. & Oberm., Sudaroli Sudha 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. To conclude, long-term exposure of UV-B radiation due to stratospheric ozone depletion can create severe suppression of nitrogen fixation activity in the root nodulating legumes.

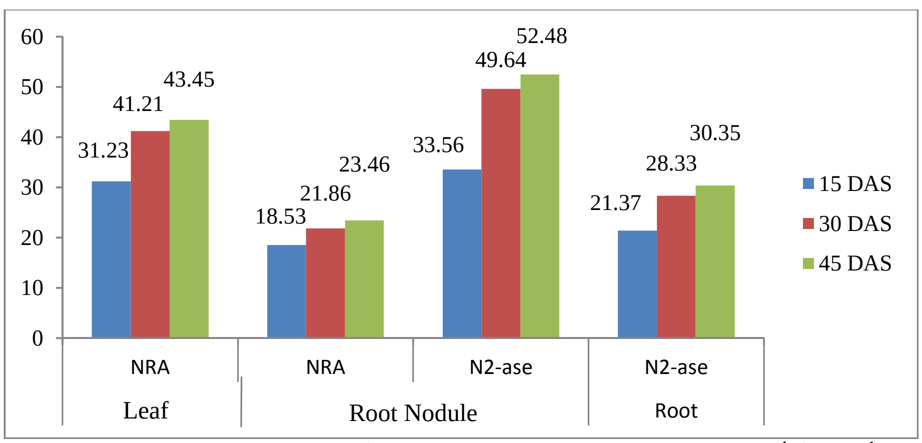

FIGURE 3: Percentage reduction in the activities of nitrate reductase, NRA [μmol(NO_2-) kg^{-1}(f.m.) s^{-1}] and in nitrogenase, N_2-ase [μmol(ethylene reduced) g^{-1}(f.m.) s^{-1}] in the 15, 30 and 45 DAS leaves, roots and root nodules of *Vigna mungo* L. var. T-9 exposed to supplementary UV-B radiation.

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