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# IMPACT OF ULTRAVIOLET-B RADIATION ON NODULATION AND NITROGEN METABOLISM IN *PHASEOLUS VULGARIS* L. C.V. PREVAIL

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## ABSTRACT
Common bean, *Phaseolus vulgaris* L. c.v. Prevail was considered as a poor nitrogen fixing plant compared to other legumes. Even though there are several attempts to produce better varieties for improved $N_2$ fixation, no work has been done to evaluate the sensitivity of this crop to elevated UV-B radiation. Depletion of ozone layer threatens to continue so as 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. As this layer cools down from the normal temperature, ozone depletion is enhanced, which an indirect effect of global is warming in addition to the direct depletion by the ozone depleting substances (ODS). As a result, the ultraviolet-B (UV-B) radiation is bound to increase, disturbing the ecosystems. In this study an attempt to assess the UV-B effects on nitrogen metabolism in the leaves, roots and nodules of common bean, *Phaseolus vulgaris* L. c.v. Prevail was made. The nodulation and nitrogen metabolism on 30 and 45 DAS (days after seed germination) of common bean 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 *Phaseolus vulgaris* L. c.v. Prevail in the leaves by 33 to 39 % and 17 to 23 % respectively and reduced nitrate and nitrite by 23 to 26 % and 36 to 38 % in the leaves and by 36 to 44 % and 46 to 48 % in the root nodules. UV-B exposure suppressed NRA (nitrate reductase activity) by 60 % in leaves and 45 to 51 % in nodules. Nodulation was suppressed by UV-B as the number of root nodules (42 to 46 %) and their fresh mass (50 %) were far below controls. UV-B stress also inhibited nitrogenase enzyme activity by 44 to 47 % in roots and by 51 to 55 % in root nodules. Present work indicates that *Phaseolus vulgaris* L. c.v. Prevail has very little tolerance to UV-B stress as indicated by the severe depression of the *Rhizobium*-legume symbiotic nitrogen fixing system.

**KEY WORDS:** Global warming, Ultra Violet-B stress, *Phaseolus vulgaris*, root nodules, nitrogen metabolism.

## INTRODUCTION
*Phaseolus vulgaris* L. c.v. Prevail (Common bean) has been considered as a poor nitrogen fixing crop compared to other legumes and only recently there have been attempts to select host plants of this species and for breeding better varieties of this crop for increased nitrogen fixation (Graham and Halliday, 1977, Fernandes *et al.* 1982). Even though there are several attempts to produce better varieties for improved $N_2$ fixation, no work has been carried to evaluate the sensitivity of this crop to elevated UV-B radiation. Human activity has dumped $CO_2$ along with other heat-trapping gases into the atmosphere which act as a blanket holding in the heat around the earth. These gases increase in thickness warming the troposphere and cooling the stratosphere thereby indirectly depleting the ozone layer in addition to the direct method by ozone depleting substances (ODS). The depletion in the ozone layer allows more ultraviolet-B (UV-B) radiation into earth's surface, affecting the growth of legumes and inhibiting biological nitrogen fixation. An elevation in the flux of ultraviolet-B radiation (280-320nm) 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 and Rajendiran and Ramanujam, 2004, Kokilavani and Rajendiran, 2013.). At the metabolism level, it severely inhibits photosynthesis (Caldwell *et al*. 1998,Kulandaivelu and Lingakumar, 2000) and hampers nodulation and nitrogen fixation (Balakumar *et al.* 1993, Rachel and Santhaguru, 1999, Rajendiran and Ramanujam 2006, Sudaroli Sudha and Rajendiran, 2013a, 2013b) in sensitive plants. Although the test plant selected for the present study was known to be a poor $N_2$ fixing crop, the objective of the present study was to find out the extent of damage caused by supplementary UV-B on nodulation and nitrogen metabolism to provide the basic information required for breeding a common bean variety with improved $N_2$ fixation as well as UV-B tolerance.

## MATERIALS & METHODS
Common bean, *Phaseolus vulgaris* L. c.v. Prevail 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 $m^{-2}$ $d^{-1}$ equivalents to 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 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 and root nodules were recorded at 30 and 45 DAS, since nodulation was at its peak level during this period. The biochemical estimations were made from the compound leaves at 30 and 45 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).

## RESULT & DISCUSSION

The protein and amino acid contents of UV-B stressed *Phaseolus vulgaris* L. c.v. Prevail, decreased by 33 to 39 % and 17 to 23 % respectively in the leaves (Table 1). According to Tevini *et al*. (1981), Vu *et al*. (1981), Rajendiran and Ramanujam (2006) and Sudaroli Sudha and Rajendiran (2013a, 2013b) reductions in soluble protein and amino acid contents of leaves are features of UV-B stress. Plants grown in controlled condition accumulated more nitrate and nitrite in the root nodules (Table 1). On the other hand UV-B stressed plants showed reduction in nitrate and nitrite by 23 to 26 % and 36 to 38 % in the leaves and by 36 to 44 % and 46 to 48 % in the root nodules respectively (Table 1).

**TABLE 1.** Changes in number and fresh mass (g) of nodules per root 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 and 45 DAS (days after seed germination) leaves, roots and nodules of *Phaseolus vulgaris* L. c.v. Prevail exposed to supplementary UV-B radiation. Means followed by different letters are significantly different at $P$ = 0.05, $n$ = 10.

| Organ | Parameter | Control | | UV - B | |
|---|---|---|---|---|---|
| | | 30DAS | 45DAS | 30DAS | 45DAS |
| Leaf | Protein | 16.83b | 20.16b | 11.22a | 12.24a |
| | Amino acid | 21.75b | 23.46b | 16.68a | 19.28a |
| | Nitrate | 4.96b | 6.23b | 3.67a | 4.79a |
| | Nitrite | 0.22b | 0.29b | 0.14a | 0.18a |
| | NRA | 2.78b | 2.89b | 1.12a | 1.16a |
| Root Nodule | Nodule Number | 26.5b | 28.5b | 14.3a | 16.3a |
| | Nodule Fresh Mass per root | 0.20b | 0.24b | 0.10a | 0.12a |
| | Nitrate | 3.32b | 3.79b | 2.11a | 2.13a |
| | Nitrite | 0.28b | 0.31b | 0.15a | 0.16a |
| | NRA | 2.32b | 2.64b | 1.26a | 1.29a |
| | $N_2$-ase | 23.10b | 25.23b | 11.27a | 11.38a |
| Root | $N_2$-ase | 0.36b | 0.38b | 0.19a | 0.21a |

Ghisi *et al.* (2002) in barley, Rajendiran and Ramanujam (2006) in *Vigna radiata* (L.) Wilczek, Sudaroli Sudha and Rajendiran (2013a) in *Sesbania grandiflora* (L.) Pers. and Sudaroli Sudha and Rajendiran (2013b) in *Vigna unguiculata* (L.) Walp. c.v. BCP-25 have reported significant reductions in nitrate reductase and glutamine synthetase activities both in the UV-B receiving leaves as well as in the root system. However 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 irradiation suppressed NRA by 60 % in leaves and 45 to 51 % in nodules. The leaves and roots of *Zea mays* L. (Quaggiotti *et al.* 2004), *Vigna radiata* (L.) Wilczek (Rajendiran and Ramanujam 2006) *Sesbania grandiflora* (L.) Pers. (Sudaroli Sudha and Rajendiran 2013a) and *Vigna unguiculata* (L.) Walp. c.v. BCP-25 (Sudaroli Sudha and Rajendiran 2013b) showed decreased values of NRA after exposure to UV-B radiation in comparison with control seedlings. 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. Guerrero *et al*. (1981) observed an accumulation of the nitrate consequent to UV-B induced inhibition of NRA, but was not confirmed by this study. Such a disparity was also reported by Balakumar *et al*. (1993) in UV-B and water stressed *Vigna unguiculata*. 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 (42 to 46 %) size and fresh mass of root nodules (50 %) were drastically reduced under controls. In contrast, nodulation and nitrogen fixation in three tropical grain legumes were not affected by exposure up to 62 % above ambient UV-B

(Samson *et al.* 2004). UV-B stress inhibited nitrogenase enzyme activity by 44 to 47 % in roots and by 51 to 55 % in root nodules. The UV-B irradiated legume on 30 as well as 45 DAS recorded reduction in all the parameters compared to control plants. However, the legume suffered heavily to UV-B on 30 DAS as the values were far below unstressed plants. To conclude, UV-B stress which was known to affect the metabolism of the aerial parts of the plants also disturbs the essential functions of the root system including *Rhizobium*-legume nitrogen fixation in *Phaseolus vulgaris* L. c.v. Prevail. As this legume was known for its poor nitrogen fixing ability, the feature of UV-B tolerance must be included while breeding an improved nitrogen fixing variety.

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