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Research Article

IMPACT OF ULTRAVIOLET-B RADIATION ON NODULATION AND NITROGEN METABOLISM IN *CYAMOPSIS TETRAGONOLOBA* (L.) TAUB. VAR. PNB

R. Vijayalakshmi and *K. Rajendiran
Department of Botany, K.M. Centre for Post Graduate Studies, Pondicherry – 605 008, India
**Author for Correspondence*

ABSTRACT
Nitrogen is a naturally occurring element that is essential for growth and reproduction in both plants and animals. It is found in amino acids that make up proteins, in nucleic acids that comprise the hereditary material and life's blueprint for all cells, and in many other organic and inorganic compounds. The legumes can convert atmospheric nitrogen to plant-available forms through a symbiotic biological process involving *Rhizobium* bacteria and the plant roots. But CO_2 and other heat trapping gases dumped into the atmosphere by human activity have increased 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 symbiotic nitrogen fixation. The present study is an attempt to assess the UV-B effects on nitrogen metabolism in the leaves, roots and nodules of cluster bean, *Cyamopsis tetragonoloba* (L.) Taub. var. PNB. The nodulation and nitrogen metabolism on 30 and 45 DAS (days after seed germination) of cluster 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 *Cyamopsis tetragonoloba* (L.) Taub. in the leaves by 33 to 38 % and 18 to 27 % respectively and reduced nitrate and nitrite by 12 to 25 % and 45 to 48 % in the leaves and by 41 to 42 % and 26 to 34 % in the root nodules. UV-B exposure suppressed NRA (nitrate reductase activity) by 58 % in leaves and 47 to 55 % in nodules. Nodulation was suppressed by UV-B as the number of root nodules (33 to 37 %) and their fresh mass (28 to 47 %) were far below controls. UV-B stress also inhibited nitrogenase enzyme activity by 29 to 32 % in roots and by 54 % in root nodules. Present study indicates that *Cyamopsis tetragonoloba* (L.) Taub. var. PNB is highly sensitive to UV-B stress and any further increase in depletion of ozone layer might enhance UV-B stress on this crop thereby severely depressing its symbiotic nitrogen-fixing ability.

Keywords: *Global Warming, Ultraviolet-B Stress, Cyamopsis tetragonoloba, Root Nodules, Nitrogen Metabolism*

INTRODUCTION
Biological nitrogen fixation is the major source of N input in agricultural soils including those in arid regions. The major nitrogen fixing systems are the symbiotic systems, which can play a significant role in improving the fertility and productivity of low-N soils. Even though behavior of *Rhizobium*-legume symbiotic nitrogen fixing system under severe environmental conditions such as salt stress, drought stress, acidity, alkalinity, nutrient deficiency, fertilizers, heavy metals, and pesticides (Hamdi 1999) have been carried out extensively, very little information is available on its UV-B tolerance. Ozone layer depletion threatens to continue so as the green house gases around the globe increase in thickness, and the heat that normally would escape the troposphere and enter the stratosphere no longer does so, leaving the stratosphere cooler. This cooling of the stratosphere enhances ozone depletion, which is an indirect effect of global warming apart from the direct depletion by the ozone depleting substances (ODS). As a result, the level of ultraviolet-B (UV-B) radiation is bound to elevate, affecting plants, animals and human beings and the ecosystems. An elevation in the flux of ultraviolet-B radiation (280-320 nm) is a harmful 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 plants generally develop tolerance to increases in UV-B flux, the objective of the present study was to find out the extent of damage caused by supplementary UV-B on nodulation and nitrogen metabolism of *Cyamopsis tetragonoloba* (L.) Taub. var. PNB, the most important food legume crop. Commonly known as cluster bean, it has high potential as a green manure and well-adapted to the drier regions, where other food legumes do not perform well.

MATERIALS AND METHODS

Cluster bean, *Cyamopsis tetragonoloba* (L.) Taub. var. PNB. 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} 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 and 45 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 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).

RESULTS AND DISCUSSION

The protein and amino acid contents of UV-B stressed cluster bean, decreased by 33 to 38 % and 18 to 27 % 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 12 to 25 % and 45 to 48 % in the leaves and by 41 to 42 % and 26 to 34 % in the root nodules respectively (Table 1). Ghisi *et al.,* (2002) in barley, Rajendiran and Ramanujam (2006) in *Vigna radiate*, 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 58 % in leaves and 47 to 55 % in nodules. The leaves and roots of *Zea mays* L. (Quaggiotti *et al.,* 2004), *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 (33 to 37 %) size and fresh mass of root nodules (28 to 47 %) were drastically reduced under controls. In contrast, nodulation and nitrogen fixation in three tropical grain legumes were not affected by exposure to 62 % above ambient UV-B (Samson *et al.*, 2004). UV-B stress inhibited nitrogenase enzyme activity by 29 to 32 % in roots and by 54 % in root nodules. Suffering under UV-B exposure, the legume on 30 as well as 45 DAS recorded reduction in all the parameters compared to same aged plants grown under normal condition. However, the legume showed severe sensitivity to UV-B stress on 30 DAS. Taken in total, it is clear that the future changes in UV-B radiation coupled with human-caused global change will have large impacts on the nitrogen metabolism in legumes, creating a threat to nutrient content of the food grains.

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 *Cyamopsis tetragonoloba* (L.) Taub. var. PNB 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	18.34b	21.67b	12.23a	13.45a
	Amino acid	25.78b	26.49b	18.63a	21.69a
	Nitrate	5.69b	6.48b	4.26a	5.71a
	Nitrite	0.29b	0.37b	0.16a	0.19a
	NRA	2.37b	2.85b	1.06a	1.18a
Root Nodule	Nodule Number	24.5b	30.5b	16.3a	19.3a
	Nodule Fresh Mass per root	0.21b	0.29b	0.11a	0.15a
	Nitrate	3.98b	4.64b	2.31a	2.74a
	Nitrite	0.23b	0.29b	0.17a	0.19a
	NRA	2.56b	2.88b	1.16a	1.53a
	N_2-ase	29.00b	31.00b	13.20a	14.45a
Root	N_2-ase	0.31b	0.38b	0.21a	0.27a

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