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BRIEF COMMUNICATION

Interactive effects of UV-B irradiation and triadimefon on nodulation and nitrogen metabolism in *Vigna radiata* plants

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Abstract

Supply of aqueous solution of triadimefon (20 mg dm^{-3}) to unstressed green gram plants increased the contents of soluble proteins, amino acids, nitrate and nitrite, and the activity of nitrate reductase in the leaves and nitrate reductase in nodules. The nitrogenase activity in nodules and roots was also increased. Number and fresh mass of nodules and their nitrate and nitrite contents were also higher than those of the controls. In contrast, the UV-B stress (12.2 kJ m^{-2} d^{-1}) suppressed nodulation and nitrogen metabolism in leaves and roots compared to plants under natural UV-B (10 kJ m^{-2} d^{-1}). Triadimefon-treated plants did not show such severe inhibitions after exposure to elevated UV-B. Thus triadimefon increased their tolerance to UV-B stress.

Additional key words: amelioration of UV-B stress, green gram, nitrate reductase, nitrogenase, triazole compounds.

Increases 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). 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) in sensitive plants. Although plants generally develop tolerance to increases in UV-B flux, it is desirable to devise chemical interventions to avoid the radiation damage. Sensitive crop species include several legumes, which are important sources of dietary proteins (Caldwell *et al.* 1998).

Triadimefon, a member of triazole family, shields the plants against biotic and abiotic stresses like ozone, sulphurdioxide, drought, salinity and chilling (Fletcher and Hofstra 1985, Muthukumarasamy and Panneerselvam 1997, Panneerselvam *et al*. 1997, 1998). Because of its ameliorative potential, it is designated as a ‘multistress protectant’ too (Fletcher and Hofstra 1985, Fletcher *et al*. 2000). Abbas and Zaidi (1997) reported that application of triadimefon alleviated UV-B-induced damages in chickpea plant (*Cicer arietinum*) and maintained the structural integrity of plasma membrane. Pursuing this lead, Rajendiran and Ramanujam (2003, 2004) further confirmed the ameliorative capacity of triadimefon in terms of growth, biomass, flowering and yield in UV-B-stressed green gram.

The objective of the present study was to find out whether the rescue potential of triadimefon could also be observed in nodulation and nitrogen metabolism of green gram since it is a nodulating grain legume.

Green gram [*Vigna radiata* (L.) Wilczek cv. KM-2] 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 by two 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 $UV\text{-}B_{BE}$ 10 kJ m^{-2} d^{-1}. Aqueous triadimefon (20 mg dm^{-3}) chemically known as 1-(4-chlorophenoxy)-3,3-dimethyl-(1,2,4-triazol-1-y1)-2-butanone was supplied through seed-soaking for 18 h, at 200 cm^3 per 100-g lot.

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Abbreviations: NRA - nitrate reductase activity; N_2-ase - nitrogenase; UV-B - ultraviolet-B radiation.
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A booster dose of triadimefon as soil drench at 30 d after sowing at 200 cm^3 per pot enhanced the effects admirably (Rajendiran and Ramanujam 2000). The seedlings (10-d-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, 45 and 60 d and the number and fresh mass of nodules were recorded. The nitrate and nitrite contents, nitrogenase and nitrate reductase activity were recorded at 30 d since nodulation was at its peak level during this period. The biochemical estimations were made from the third trifoliate leaves at 30 d. 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).

Triadimefon application increased the protein and amino acid contents of green gram by 32 and 33 % respectively which declined sharply in UV-B stressed plants by 34 % (Table 1). Plants preconditioned with triadimefon also had higher protein and amino acid levels despite exposure to elevated UV-B radiation but were still less than the controls. Increased protein and amino acid contents observed in this study were also reported in triadimefon treated-soybean seedlings (Panneerselvam *et al*. 1998) and cowpea (Gopi *et al.* 1998). Reductions in soluble protein and amino acid contents of leaves are features of UV-B stress (Tevini *et al*. 1981, Vu *et al*. 1981). Triadimefon enabled the UV-B stressed plants to elevate these metabolites to the level of control. Whereas an increase in protein content points to an acceleration of protein synthesis, the decreases could be due to inhibition of its synthesis, accelerated proteolysis, decreased availability of amino acids or denaturation of enzymes (Levitt 1972). The free amino acid content, which declined under UV-B, was increased by triadimefon, thereby facilitating the levelling off in the combined treatment. Maintaining a larger amino acid pool appears to be characteristic of triadimefon as observed in soybean (Panneerselvam *et al*. 1998) and cowpea (Gopi *et al.* 1998).

Unstressed plants treated with triadimefon accumulated more nitrate and nitrite by 15 and 5 % in leaves and by 9 and 8 % in the nodules (Table 1). This is in accordance with the results of Srivastava and Fletcher (1992) who reported enhanced nitrate uptake and NRA in *Canola* leaves after triadimefon application. However, the contents of nitrate and nitrite were suppressed by UV-B stress by 22 and 28 % in leaves and 16 and 23 % in the nodules, respectively. Similar reductions were earlier reported by Balakumar *et al*. (1993) in *Vigna unguiculata* and *Vigna radiata*, and in *Vigna mungo* by Rachel and Santhaguru (1999). Ghisi *et al.* (2002) observed significant reductions in the activities of nitrate reductase and glutamine synthetase in barley, 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. In UV-B stressed plants, triadimefon restored the foliar and nodular nitrate and nitrite contents on par with controls (Table 1).

Table 1. Changes in 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 d leaves, roots and nodules of green gram exposed to supplementary UV-B radiation, aqueous triadimefon (TRIA, 20 mg dm^{-3}) and their combinations. Means followed by different letters are significantly different at P = 0.05, n = 10.

Organ	Parameter	Control	UV-B	TRIA	UV-B+TRIA
Leaf	protein	18.04b	11.88a	23.91c	18.01b
	amino acid	24.22b	16.02a	32.19c	24.18b
	nitrate	4.42b	3.46a	5.07c	4.23b
	nitrite	0.21b	0.15a	0.22b	0.19b
	NRA	1.46b	0.98a	1.81c	1.45b
Nodule	nitrate	3.64b	3.05a	3.96c	3.51b
	nitrite	0.26b	0.20a	0.28b	0.25b
	NRA	2.17b	1.78a	2.80c	2.14b
	N_2-ase	30.00b	10.00a	48.00c	29.00b
Root	N_2-ase	0.40b	0.30a	0.43b	0.38b

Triadimefon supply to unstressed plants favoured NRA both in the leaves by 24 % and the nodules by 29 % over control plants (Table 1). On the other hand UV-B exposure suppressed NRA by 33 % in leaves and 18 % in nodules. 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). 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 occurred in UV-B stressed *Vigna unguiculata* also (Balakumar *et al*. 1993). According to Ghisi *et al*. (2002), nitrate content of neither the leaf nor root was influenced by elevated UV-B. However, triadimefon treatment helped the plants under UV-B stress to elevate their NRA to the control level both in the leaves and the root system (Table 1).

Triadimefon exerted a stimulatory effect on nodulation; the nodules were more in number (24 to 29 %), larger in size and heavier (23 to 29 %) (Fig. 1). Nodulation was inhibited severely by UV-B as the number (41 to 49 %), size and fresh mass (42 to 45 %) were far below controls. In contrast, nodulation and

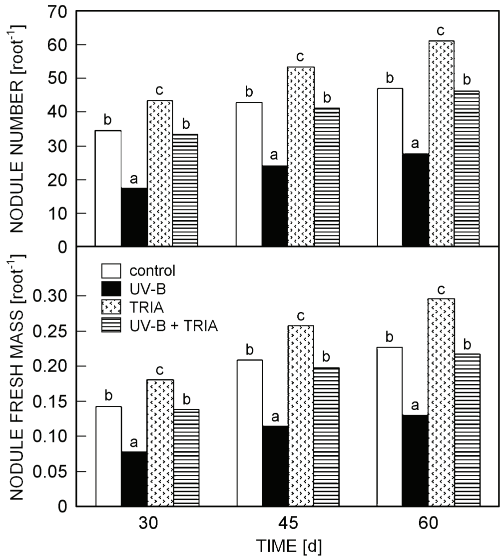

Fig. 1. Changes in the number and fresh mass of nodules per root system of green gram exposed to supplementary UV-B radiation, aqueous triadimefon (TRIA, 20 mg dm^{-3}) and their combinations. Bars having different letters are significantly different at $P = 0.05$, $n = 10$.

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). With reference to Fig. 1, under the influence of triadimefon, even the UV-B stressed plants produced more and heavier nodules, but just below the control plants.

Without UV-B stress, triadimefon increased nitrogenase activity only marginally in the roots whereas it was significantly higher (60 %) in the nodules (Table 1). UV-B stress inhibited this enzyme activity by 25 % in roots and by 67 % in nodules. Preconditioning with triadimefon proved beneficial as it abolished the inhibitions by 20 % in roots and by 64 % in nodules, thereby maintaining normality in symbiotic nitrogen fixation.

In short, triadimefon appears to be effective in minimising the damages to nitrogen metabolism induced by UV-B stress. Since elevated UV-B continues to be a critical environmental stress, the ameliorative potential of triadimefon is quite promising in the context of food and nutrient security.

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