Asian J. Exp. Sci., Vol. 21, No. 1, 2007, 109-114

UV–β, Stress and Triademefon Induced Effect on Nitrogen Metabolism and Anti-oxidative Enzymes of *Lablab purpureus* Seedlings

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Abstract : The role of antioxidative enzymes in protection against UV–β (280-320 nm) stress was studied in *L. purpureus* seedlings at 5 days after germination. Treatment of triademefon alone and in combination with UV–β was given in *L. purpureus* seedlings grown *in vitro* for soluble protein, proline contents and alanine amino transferase (ALT), aspartate amino transferase (AST), nitrate reductase (NR), superoxide dismutase (SOD) and peroxidase (POX) activities. Treatment of triademefon – a triazole plant growth regulator had negative impact on protein, proline contents and enzymatic activities of ALT and NR. However, activity of AST was found to be increased with triademefon under UV–β stress. The increased activities of SOD and POX participated in enhanced tolerance to oxidative damage under UV–β stress. Thus, triademefon can alleviate the effect of UV–β stress in *L. purpureus* seedlings by enhancing the activities of antioxidative enzymes like SOD and POX.

Key words : Alanine amino transferase, Aspartate amino transferase, Nitrate reductase, Peroxidase, Superoxide dismutase.

Introduction :

UV–β (280-320 nm) radiation reaching the earth's surface increasing because of depletion of the stratospheric ozone layer (Kerr and McElory, 1993; Stolarski *et al.*, 1992). Increased UV–β radiation has been reported to cause detrimental effects on defferent plant species (Hemantaranjan, 2006) among which inhibition in the growth and photosynthesis are the basic responses to enhanced UV–β radiation (Teramura, 1983 and Sulvian, 1994). Some plants have various defense mechanisms in response to UV–β radiation (Beggs *et al.*, 1986; Cuin, 2006; Tevini *et al.*, 1991). The deleterious effects of UV–β on plants have been extensively studied (Teramura and Sulivan, 1994). The role of free radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) has been identified as a major biochemical mechanism to prevent harmful effect of photo-oxidation in plant cells exposed to UV–β radiation.

Triazoles are group of PGRs which include wide variety of responses in plants, such as reduced sterol biosynthesis, altered ABA contents, increased chlorophyll contents, altered carbohydrate status and increase stress tolerance (Davis *et al.*, 1988). Triademefon which is a triazole is a highly active systemic fungicide.

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Among certain crop legumes, *Lablab purpureus* is a much economic importance and used as food and fodder. Therefore, the present investigation was carried out to evaluate the performance of *L. purpureus* seedlings and the role of triademifon in improving the tolerance to UV–β stress.

Material and Methods :

Viable seeds of *L. purpureus* (L.) var. L.P.-1461 were selected for this investigation. The seeds were sterilized by treating them with 0.1% mercuric chloride ($HgCl_2$) solution for 5 minutes and thoroughly washed with doubly distilled water 4-5 times. The sterilized seeds were then germinated in petridishes lined with Whatman filter paper and were kept at 28 ± 2° C in BOD incubator. A cool fluorescent light of 34.1 mol m^{-2} $sec.^{-1}$ PAR was given to the seeds. There were three applications for stressed and unstressed seedlings taken for analyzing different parameters.

For UV–β studies seeds were germinated in 9 cm petriplates lined with double layer of filter paper. These petriplates were allowed to germinate with respective solution for 5 days in complete darkness at 28 ± 2° C. Cotyledons were excised from 5 days old seedlings in dark under green safe lamp (Philips 25 W, covered with 8 layer of cellophone). Excised cotyledons were exposed to UV–β (280-320 nm] irradiation for 30 min. before analysis. Irradiation at the level of seedlings were 2.66 mw cm^{-2} sec^{-1}. Seeds were subjected to UV–B radiation alone and also in combination with triademefon.

The seedlings were analyzed on 5 days after germination for soluble protein, proline and enzymatic activities. Soluble protein were estimated colorimetrically using the coomasive blue dye – binding method by Bradford (1976) and free proline by Bates *et al*. (1973). The activities of alanine amino transferase (ALT) and aspartate amino transferase (AST) were assayed by Bergmeyer's method (1974). Nitrate reductase (NR) was assayed following Wray and Filner (1970) method, superoxide dismutase activity was determined according to the method of Beauchamp and Fridovich (1971) with some modification (Gannopolitis and Ries, 1977) and peroxidase activity was determined by the method of Honold and Stahmann (1968).

Results and Discussion :

In vitro studies conducted in *L. purpureus* seedlings exhibited decreased protein content when treated with UV–β radiation alone and also declined when the radiation was given in combination with triademefon (Table 1). When compared to control growth seedlings. The depletion of protein indicates the possibility of the high rate of utilization of proteins by the seedlings for their metabolic activities under UV–β stress or by the lower activities of amino transferases. Similarly, decrease in protein content under UV–β exposure was observed in cucumber cotyledons (Takeuchi *et al.*, 1989) and in mung bean plant (Pal *et al.*, 1999). .

Proline is an important metabolic constituent and can easily be converted to key amino acids, glutamate. It has been established fact that proline accumulates

in excised and intact plant part under abiotic stress. It has been suggested the proline protects plant tissue against osmotic stress because of osmo solute, a source of nitrogen structures (Steward and Lee, 1974; Chakraborty *et al.*, 2005). Recently it was reported that constitute production of proline could confer osmo tolerance in transgenic tobacco plants (Kishore *et al.*, 1995). But in present study the proline content was found to be decreased with triademefon under UV–β stress in *L. purpureus* seedlings (Table 1).

Aminotransferases (ALT and AST) play an important role in distribution of the assimilated nitrogen in to protein amino acids during transamination reaction. In higher plants the trans amination reactions are responsible for the synthesis of all amino acids that finally incorporates into proteins. Moorthy and Kathiresan (1997) reported high dose of UV–β on phenolic compounds, amino acids, lipids, saccharides and proline. In present investigation, treatment of triademefon inhibited ALT and AST activities but in combination with UV–β decreased ALT activity and increased AST activity when compared to control (Table 2). The decreased activity of ALT could either be due to lowering of synthesis of glutamate or pyruvate or might be due to the fact that active sites of ALT were blocked by an inhibitor so that the product that is amino acid alanine could not be formed.

The reduction of nitrate to nitrite catalysed by nitrate reductase (NR) is considered to be the rate limiting step of N-assimilation. NR activity in *L. purpureus* grown in *in vitro* seedlings were found to be inhibited with triademefon and also under UV–β stress (Table 2). Similarly, Gadi and Bohra (2001) reported decreased NR activity in triademefon treated Zizyphus *mauritiana* Var. *rotundifolia* seedlings. Thus, it is clear from the results that triademefon acts as inhibitor for the activities of enzymes of N-metabolism.

It is evident from the present investigation that treatment of triademefon had inhibitory effect on the metabolites and enzymatic activities of nitrogen metabolism in *L. purpureus* seedlings under UV–β stress. Still the plant is surviving may be due to strong antioxidant defense system in *L. purpureus* seedlings. But, further investigation on other enzymes is needed on nitrogen metabolism under UV–β stress before conclusions can be drawn.

On the other hand, triademefon treated *L. purpureus* seedlings under UV–β stress proved to be beneficial on the activities of antioxidative enzymes like superoxide, dismutase (SOD) (Kasturibai and Aziz, 2004) and peroxidase (POX). The activities of SOD similar as in cucumber cotyledons (Sunita and Guruprasad, 1998) and POX were enhanced in *L. purpureus* seedlings with both the concentration of triademefon under UV–β stress (Table 2). Similarly the activity of SOD was increased in cucumber seedlings exposed to 6 hr. of UV–β radiation (Noriaki and Mika, 2000) and in wheat (Dawar *et al.*, 1998). The peroxidase activity was also reported to be increased by UV–β irradiation in *Hibiscus rosasinensis* (Panagopoulos *et al.*, 1989), *Beta vulgaris* (Panagopoulos *et al.*, 1990)

Table 1 : Effect of triademefon along with UV-B exposure on soluble protein and proline contents in *L. purpureus* seedlings grown *in vitro*.

Treatments	Soluble protein (mg g^{-1} FW)	Proline (m mol g^{-1} FW)
Control	65.353 ± 0.083	1.173 ± 0.038
Triademefon (5 mg/l)	60.016 ± 0.127	0.653 ± 0.033
Triademefon (20 mg/l)	55.828 ± 0.085	0.538 ± 0.013
UV-β (30 min)	59.179 ± 0.058	0.755 ± 0.033
Triademefon [5 mg/l + UV-β (30 min)]	57.612 ± 0.043	0.638 ± 0.057
Triademefon [20 mg/l + UV-β (30 min)]	56.432 ± 0.069	1.165 ± 0.064

± = values represent S.D. of three replicates.
UV-β = Ultra violet – β

Table 2 : Effect of triademefon along with UV-β exposure on the activities of amino transferases (ALT, AST), nitrate reductase (NR), superoxidase dismutase (SOD) and peroxidase (POX) in *L. purpureus* grown *in vitro*

Treatment	ALT (μ mol mg^{-1} protein min^{-1})	AST (μ mol mg^{-1} protein min^{-1})	NR (n mol mg^{-1} protein min^{-1})	SOD (n mol mg^{-1} protein)	POX (mg g^{-1} FW min^{-1})
Control	3.706±0.026	1.944±0.012	7.944±0.018	2.098±0.489	7.339±0.110
Triademefon (5 mg/l)	2.148±0.026	1.572±0.005	2.228±0.041	8.148±0.234	10.834±0.020
Triademefon (20 mg/l)	2.617±0.089	1.793±0.064	1.856±0.187	13.969±0.156	14.496±1.098
UV-β (30 min)	1.746±0.022	2.6120.018	3.777±0.072	1.936±0.338	15.149±0.840
Triademefon [5 mg/l + UV-β (30 min)]	2.252±0.033	2.315±0.094	4.484±0.062	12.549±0.880	23.846±1.143
Triademefon [20 mg/l + UV-β (30 min)]	2.356±0.041	2.491±0.014	2.890±0.053	14.936±0.739	25.440±0.236

± = values represent S.D. of three replicates.
UV-β = Ultra violet – β

and *Cucumis sativus* var. long Green (Tekchandani and Guruprasad, 1998).

It is apparent that not only superoxide radical scavenging enzyme SOD is important but also H_2O_2 scavenging enzyme POX is equally important in imparting tolerance against UV–β stress (Kasturibai and Aziz, 2004). Chakraborty and Chakraborty (2005) have reported alteration in the metabolic process during abiotic stresses.

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