

Regular Article

Impacts of ultraviolet-B radiation on Antioxidant defense system in *Aeschynomene aspera* L.

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Sunlight contains a small amount of short wavelength ultraviolet (UV) light irradiation, which is harmful to life on planet earth. Penetration of ultraviolet radiation varies among different plant species and may reflect their sensitivity. The effects of UV-B radiation was studied in *Aeschynomene aspera* L. The selected plant was exposed to UV-B radiation for 9 days and determined the changes. Plants protect themselves from this harmful radiation by altering the antioxidant enzymes. Specific enzyme activity of polyphenoloxidase was decreased. In contrast to the polyphenoloxidase enzyme, catalase, peroxidase and phenylalanine ammonia- lyase were found to be increased. Proteins were accumulated on the 9th day of UV exposure and it was confirmed from the SDS-PAGE by the banding pattern of protein. The result shows that *Aeschynomene aspera* L. developed resistance against UV-B radiation by inducing the proteins and antioxidant defense system.

Keywords: UV-B radiation, *Aeschynomene aspera*, polyphenoloxidase, catalase, peroxidise.

Ozone is an important minor constituent of the atmosphere. It is found at altitudes between 10 and 30 kilometer with a maximum concentration from 19 to 23 kilometer. But this protective shield is being continually damaged by human activities. Usually, UV radiation is divided into UV-A (315 to 400 nm), UV-B (280 to 315 nm) and UV-C (100 to 280 nm). UV-C radiation is completely absorbed by the ozone layer while UV-A radiation is unaffected and does not harm plants. UV-B radiation intensity alters the plant ecosystems by reducing the productivity of several economically important crops. UV-B radiation causes mutations in plants by promoting the formation of cyclobutane pyrimidine dimmers between adjacent pyrimidine bases (Britt, 1996). Within the UV-B spectral region, the relative

enhancement in irradiance resulting from ozone depletion is disproportionately greater at shorter wavelengths (Caldwell *et al.*, 1998). Numerous studies have investigated the effects of elevated UV-B on plants, and have shown a diverse range of responses, including changes at the physiological, morphological, biochemical and molecular levels (Paul, 2001). The number of studies on the effects of UV-B radiation, especially on plants and insects were increased in the last twenty years, with most studies artificially enhancing UV-B radiation by using UV-B lamps under controlled conditions, e.g. in greenhouses or climate chambers (Wei Gaoa *et al.*, 2003; Feng *et al.*, 2003;), or in field experiments (e.g.; Bjerke *et al.*, 2005) in order to test the effects on plants and their potential for adaptation and self-protection. UV-B

radiation affects the terrestrial plants through changes in plant growth, photosynthesis transpiration, yield, susceptibility to disease, environmental stress and pollution. It is well established that a major site of damage by UV-B is the chloroplast, leading to impairment of photosynthetic function (Bornman, 1989). Some plants are more tolerant to UV-B because they produce a variety of secondary metabolites that effectively absorb UV-B and prevent it from penetrating in to the leaf mesophyll cells. Plants have developed a complex antioxidant system that includes reduced glutathione (GSH), ascorbic acid, carotenoids and other enzymes that protect the plant against oxidative damage. Antioxidants occur naturally in plants. It include phenolic compounds and anthocyanins. Phenolics may be of vital importance to plants exposed to excess solar high frequency radiation. The biosynthesis of phenolics in plants is activated by enhanced UV-B (280-320 nm) light (Tegelberg and Julkunen-Tiitto, 2001).

Teramura *et al.* (1983) reported the interveinal wrinkling and leaf chlorosis observation in UV-B treated plants. The enhanced UV-B radiation activates the antioxidant enzymes and induces polyamines but also cause damage in the leaves, exemplified by an increase in polyphenol oxidase activity and a decrease in chlorophyll concentration. Hiraga *et al.* (2000) stated that SOD is the first line of detoxifying enzymes, could scavenge ROS enzymatically, that is, it converts O_2 to a less toxic ROS (H_2O_2). Ascorbate peroxidase (APX; EC 1.11.1.11) degrades H_2O_2 via AsA oxidation, and dehydroascorbate (DHA, oxidized AsA) is reduced to regenerate AsA by dehydroascorbate reductase (DHAR; EC 1.8.5.1) using GSH as the electron donor. Guaiacol peroxidase (G-POD) and ascorbate peroxidase (AsA-POD) are peroxidase enzymes that are found in animal, plant and microorganism tissues, which can catalyze oxidoreduction between hydrogen peroxide (H_2O_2) and various

reductants. The plant material selected for the study is *Aeschynomene aspera* L. Belonging to family Fabaceae. It is a tall erect sub shrub in swampy areas, with stout nodular stems. *Aeschynomene aspera* is propagated by seeds and stem. It is also known by the names Sola, Sola Pith Plant, Pith Plant, Laugauni or Netti. The aim of our work was to investigate the effects of antioxidant enzymes in *Aeschynomene aspera* L. and to determine the banding pattern of proteins by using SDS PAGE.

Materials and methods

Collection of plant sample

Aeschynomene aspera seeds were collected from Tamil Nadu Agricultural University Coimbatore. Seeds were soaked in water for one day and then transferred to garden soil in trays, containing red earth, sand and farmyard manure in (1:2:1). UV-B radiation was provided by UV lamps which are above 15cm and control is maintained. Control was exposed to normal day light. Seedlings were irradiated for 1 hour per day (12.30 pm to 1.30 pm) for 9 days.

Estimation of antioxidant enzyme activity

Catalase enzyme activity

Catalase activity (CAT, EC 1. 11.1.6) of leaves was determined by following the method of Luck (1974). One gram of the sample was extracted in 10ml of 0.067 M phosphate buffer (pH 7.0) and centrifuged at 12,000 g for 10 minutes. The supernatant was used for assay. A known volume of the extract was added to the experimental cuvette containing 3 ml H_2O_2 - PO_4 buffer (0.8 ml of H_2O_2 in 49.2 ml of PO_4 buffer). The time taken for percent change in absorbance (Δt), at 240 nm was recorded for calculating the enzyme activity and expressed as enzyme units/gm fresh tissue.

Peroxidase enzyme activity

Peroxidase (EC1.11.1.7; donor: hydrogen-peroxidase oxido-reductase) enzyme was assayed as per the procedure described by Putter (1974), with slight modifications. 500 milligram fresh leaves were homogenized with 0.1M Phosphate

buffer (pH 7) and centrifuged at 10,000 g for 10 minutes. The supernatant was used for assay. The reaction mixture consisting 0.05 ml of 20mM Guaiacol in 3ml of 0.1M Sodium phosphate buffer, pH 6.0 and 0.03 ml of 0.1M Hydrogen Peroxide. Enzyme extract (0.1 ml) was added to initiate the reaction. The absorbance change was recorded at 430 nm. The reagents without the enzyme extract were served as blank. Peroxidase enzyme activity was expressed as change in absorbance at 430 nm per units/g fresh tissue.

Polyphenoloxidase enzyme activity

Polyphenoloxidase (EC 1.10.3.1) activity was assayed by the method of Kumar and Khan (1981) with slight modifications. 500 milligram fresh leaves were homogenized with 0.1M Phosphate buffer (pH6.8) and centrifuged at 12,000 g for 10 minutes. The supernatant was used for assay. Polyphenoloxidase was determined by measuring the assay mixture contained 2ml of phosphate buffer (pH 6.0) 1ml of 0.1M catechol and 0.5 ml of enzyme extract. This was incubated for 5 minutes at 25°C, and then the reaction was stopped by adding 1ml of 2.5 N sulphuric acids. The absorbance of was recorded at 495nm. The reaction mixture without the enzyme extract is used as blank. Enzyme activity was calculated from the formula.

Phenylalanine ammonia- lyase enzyme activity

Phenylalanine ammonia- lyase (EC 4.3.1.5) was determined by following the method of Bruseke (1980). 500 milligram fresh leaves were homogenized with Phosphate buffer (pH 8.7) and centrifuged at 12,000 g for 10 minutes. The supernatant was used for assay. An aliquot of 0.2 ml of enzyme extract was added with 0.5 ml borate buffer and 1.3 ml distilled water. The reaction of was initiated by adding 1ml of 0.1 m phenylalanine solution and incubated for 30-60 minute at 32°C. After incubation the reaction was terminated by adding 0.5 ml of 1M trichloroacetic acid and measured the absorbance at 290 nm, against blank.

Estimation of Protein:

The protein content was determined by Lowry *et al.* (1951) method. 0.5 gm of leaves was smashed with 80% Acetone. This extract was used as a sample. 0.3 ml of extract was diluted to 1 ml. Then 2 ml of Reagent D was added to it and incubated it for 10 minutes at room temperature. 0.6 ml of Reagent E was added and and incubated it for 20 minutes at room temperature. Absorbance was read at 660nm with bovine serum albumin as a standard.

Specific enzyme activity

For Antioxidant enzymes Catalase, Peroxidase, Polyphenoloxidase, Phenylalanine ammonia- lyase Specific enzyme activity was calculated using protein concentration.

SDS-PAGE

Extraction of Proteins:

Leaf disks were ground at 4 °C in Tris-HCl (60 mM, pH6.8) using a pre-chilled mortar and pestle. The extract was centrifuged (15 min at 4000 × g) and the supernatant used for protein quantification (soluble protein) according to Peterson (1977). The pellet was re-suspended in the same buffer containing sodium dodecyl sulphate (SDS) 2.5% (w/v) in order to extract the insoluble proteins (suspension incubated for 3 h at 4°C followed by 1 h at room temperature). The suspension was then centrifuged for 15 min at 4000 × g and proteins quantified via the same method.

Results and Discussion

Antioxidant enzyme activity

Antioxidant enzymes and their activities of involved in Reactive oxygen species metabolism. In the present study antioxidant enzymes such as catalase, peroxidase, polyphenoloxidase and phenylalanine ammonialyase specific enzyme activity were determined from their enzyme activity. The antioxidative capacity also altered in experimental plants under UV-B radiation.

Catalase is the most efficient antioxidant enzyme which protects plants

by scavenging free radicals and H₂O₂. Compared to control catalase does not showed significant activity on the 6th day but activity was increased and on 6th day specific enzyme activity was (37.2%).

Specific enzyme activity of Catalase was shown in the figure 1. Inhibition of catalase activity was due to enzyme consumption to detoxify H₂O₂ or enzyme inactivation (Ambasht and Agrawal, 2003).

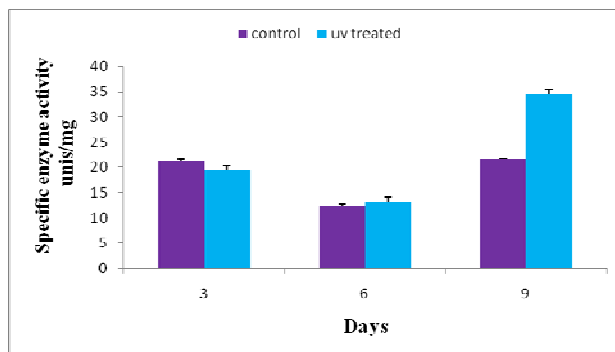


Figure:1 Effect of UV-B radiation on specific enzyme activity of catalase

Along with catalase activity, peroxidase activity was also an important component of antioxidant defense system for scavenging H₂O₂. Guaiacol was used as substrate and due to peroxidase activity in the presence of H₂O₂ it was converted into oxidized guaiacol. Peroxidase activity was gradually increased after the UV treatment and highest increase about (84.5%) and shown in the figure 2.

compound. Specific enzyme activity of polyphenol oxidase was inhibited under UV-B treatment and the gradual decrease in activity was observed about (30.4%). Similar to peroxidase, phenylalanine ammonia-lyase activity was also increased under supplemental UV-B radiation. Studies in cucumber have shown that exposure of seedlings to supplemental UV-B radiation caused 78 percent increase in the activity of phenylalanine ammonia-lyase (Krizek *et al.*, 1993).

Polyphenol oxidase is also responsible for the oxidation of phenolic

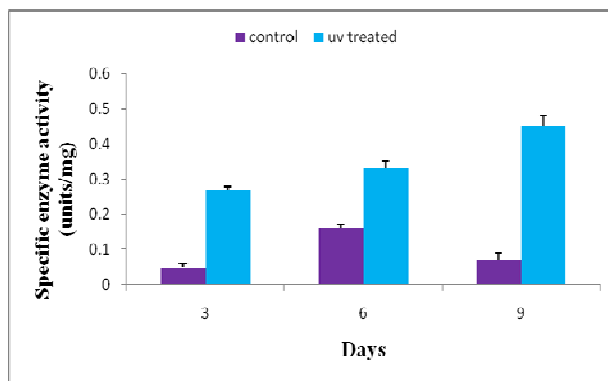


Figure 2: Effect of UV-B radiation on specific enzyme activity of peroxidase

Banding Pattern of Proteins

SDS-PAGE was carried out only after 9 days of UV-B treatment. This was due to increase in protein content was found in UV-B treated plants on their 9th

day. This result was observed from protein quantitative estimation. SDS PAGE was carried out with 8% separating gel and 4% stacking gel. Proteins were extracted and soluble proteins were loaded in the well.

From the banding pattern of protein it was observed that Lane 2 showed high protein content than Lane 1. Pruvot *et al.* (1996) suggested that drought-induced stress protein participates in the stabilisation of

thylakoids preventing damage resulting from osmotic or oxidative stress. The analysis of electrophoresis pattern of soluble proteins were shown in the figure 5.

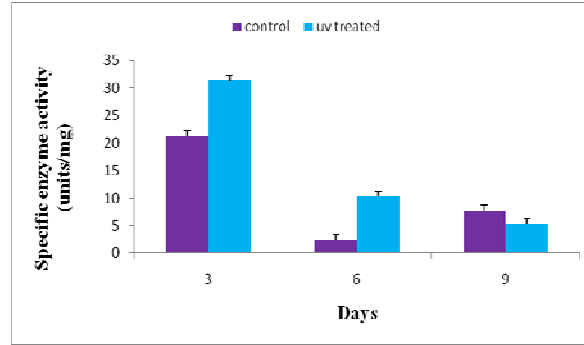


Figure 3: Effect of UV-B radiation on specific enzyme activity of polyphenol oxidase

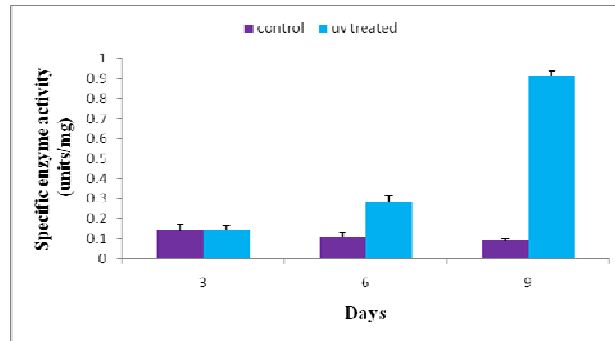


Figure 4: Effect of UV-B radiation on specific enzyme activity of phenylalanine ammonialyase

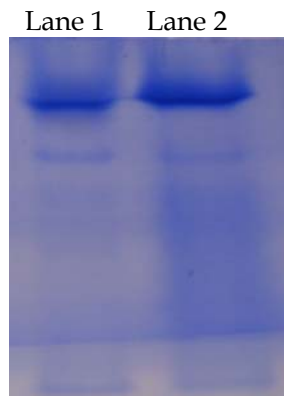


Figure 5: SDS PAGE showing the protein band of control and UV-B treated plant on the 9th day; Lane Description: Lane 1: Control, Lane 2: UV treated.

Conclusion

Thus it can be concluded that after nine days of treatment, *Aeschynomene aspera* L. exhibits a different sensibility to UV-B radiation. UV-B adversely affected the antioxidant enzyme activity. Thus the accumulation of proteins and antioxidant enzymes provide protection to the plant during oxidative stress.

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