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Studies on Superoxide Dismutase Activity under SO_2 Fumigation in Vigna Unguiculata Leaves

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Abstract: ***The aim of the present study was to investigate the effect of sulphur dioxide (SO_2) on superoxide dismutase (SOD) activity at different stages of leaf development in Vigna unguiculata (V. unguiculata) leaves. V. unguiculata plants were fumigated with SO_2 at 0.1 ppm and 0.2 ppm concentrations for 20 days. After 20 days of fumigation, leaves at different stages were taken for the study. Leaf injury and SOD activity were analysed. Increased SOD activity was observed at 0.1 ppm of SO_2. Leaf injury was more in fully expanded mature leaves when compared to younger leaves and control leaves. SOD activity was more in younger leaves than in mature leaves which were treated with SO_2 fumigation. In conclusion, V. unguiculata plant tissues has its own defense mechanism and scavenging efficacy against toxic free radicals.***
Keywords: Free radicals, Scavenging enzyme, SO_2, Superoxide dismutase, Vigna unguiculata

I. INTRODUCTION

The health and welfare of mankind is intimately linked with the viability and productivity of natural and agricultural ecosystems. The problems of atmospheric pollution are rapidly growing and are being viewed seriously in India. The steadily increasing demand for energy and other natural resources are found to the main cause of air pollution. The phenomenon is particularly associated with ecologically unplanned industrialization and uncontrolled urbanization [1].

SO_2 is one of the major air pollutants, which causes serious damage to vegetation. SO_2 is emitted into the atmosphere by combustion of sulphur containing mineral ores [2]. The most important phytotoxic air contaminants are generally gaseous in nature. SO_2 is toxic to vegetation even at relatively lower concentration (less than 1 ppm). Among the various living components of ecosystem, plants are found to be more sensitive the toxic effects of air pollutants [3]. When plants grow in an environment polluted with SO_2, it gets entry into the leaf tissue through stomata and subsequently produces H^+, HSO_3^-, and SO_3^{2-} in the cells. Formation of these derivatives causes many toxic effects in plant tissue [4].

The presence of sulphite or bisulphite leads to a free radical chain reaction generating increased superoxide ions and other reactive oxygen species can cause oxidation of various cellular components with consequential damage to plants [5]. Most of the HSO_3^- and SO_3^{2-} get photooxidized to the less toxic SO_4^{2-} in chloroplasts, along with the production of O^{2-} (superoxide radical) which increases the formation of oxygen free radicals in the chloroplasts. This active oxygen is highly reactive with various cell components and its accumulation cause oxidative damage to plants [6].

SOD has been examined in photosynthetic organisms [7]. It is an enzyme responsible for th breakdown of O_2^-. It is a metalloprotein which catalyzes the dismutation of O_2^- to O_2 and H_2O_2 by altering the concentration of O_2^-, SOD helps to prevent both direct toxicity from O_2^- and secondary toxicity from H_2O_2 [8]. SOD inhibits the chain oxidation of sulphite by scavenging O_2, thus it is one possible tolerance mechanism of leaf cells against SO_2 [9]. A large volume of literature exists describing various physiological and biochemical effects of fluoride on higher plants. In the present study, an attempt has been made to find out the effects of SO_2 on SOD activity in V. unguiculata L. Walp.

II. MATERIALS AND METHODS

A. Plant Materials

Seeds of Vigna unguiculata L. Walp. CV. 152 were obtained from Tamilnadu Agricultural University, Coimbatore, India. Seeds were surface sterilized with 0.1 % mercuric chloride and soaked in water for 10 – 20 h and sown in prepared pots of 12 cm diameter and maintained under natural conditions at the Botanical Garden, Maduari Kamaraj University, and Madurai. The plants were periodically 7000 – 8000 µ E m^{-2}-S^{-1} light intensity with 12 h D/12 h L photo period and temperature of 37 ^oC (day) and 28 ^oC (night). The plants were periodically watered and care was taken to avoid any mineral deficiency and microbial contamination. Extra seedlings were thinned after 10 days of growth and only 6 healthy seedlings were allowed to grow in each pot for further studies.

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B. Determination of LD_{50}

Plants were fumigated with various concentrations of SO_2 ranging from 0.1 – 1.0 ppm of SO_2. A 50% reduction in growth was observed at 0.25 ppm of SO_2. Therefore 0.1 ppm and 0.2 ppm concentrations were taken as experimental treatments for further studies. The SO_2 fumigation (2h/day) was started from 16th day of plant age and continued till 45 days of plant age (20 days of SO_2 exposure). A control was maintained under identical environmental conditions.

C. Fumigation Technique

Fumigation was performed in a closed chamber (50*75*75 cm), which was fabricated by transparent PVC sheet. Experimental pots were kept in the fumigation chamber and SO_2 was generated in a continuous manner by bubbling and through aqueous sodium metabisulphite solution and desired concentration within the chamber was achieved through dilution with carrier air at a flow of 1.46 m^3/min. The gas was uniformly distributed in the fumigation chamber through a network of perforated alkaline pipes arranged at the base. SO_2 within the chamber was monitored as follows.

100 ml of 1 % aqueous sodium metabisulphite was prepared in 250 ml of round bottom flask and the flow rate of air was maintained at 1.5 L/min. The plants were fumigated for a set period and subsequently the gas was collected in 10 ml of sodium tetra chloro mercurate (II) solution (0.1 mole mercuric chloride, 27.2 g and 0.2 mole of sodium chloride, 11.7 g dissolved in water and diluted to 100 ml) in a closed container connected through a small frilled scrubber. To this reaction solution, 1 ml of acidic 0.04 % p-rosaniline hydrochloride and 1 ml of 0.2 % formaldehyde solution were used and this solution was allowed to sand for 20 – 30 min for full color development. A blank was maintained with 10 ml of sodium tetra chloro mercurate (II). Absorbance of the test solution was determined against blank at 560 nm. Concentration of SO_2 was calculated from a standard curve prepared by using standard solution of sodium sulphite in sodium tetra chloro mercurate (II). Each µg of SO_2 represented 0.1 ppm of SO_2 in the exposures [10].

D. Sample Preparation and SOD assay

After fumigation, fresh leaves were harvested at 5 days intervals and used for further analysis. Fresh leaves (10 g) were homogenized in 0.05 M sodium phosphate buffer (pH 7.3) with 0.6 g insoluble polyvinyl pyrolidine (PVP) and 2 g acetone powder. The suspensions were centrifuged at 13,000 rpm for 15 min at 4 ^oC. Residues were reextracted twice by resuspension and centrifugation in 5 ml portions of phosphate buffer. Combined supernatants were brought to final sample volumes of 30 ml. Extracts at this stage were used for the estimation of SOD activity described by Mc Cord and Fridovich [11] following the photoreduction of ferricytochrome C. The control rate was adjusted to 0.025 A (at 550 nm) per min at room temperature by adding 0.033 unit xanthine oxidase solution to the reaction mixture. The rate of reaction was read at 15 seconds intervals for 1-2 min. One unit of SOD activity was defined as that which inhibited 50 % of the reaction rate under these conditions.

III. RESULTS AND DISCUSSION

A. SO_2 exposure and SOD activity

SOD is the first line of defense against oxy-radical mediated injury. To defend themselves against oxidative stress, most plants have effective decontamination systems and are equipped with various antioxidants [12]. Changes in the level of SOD activity at different concentration of SO_2 fumigation in *V. uniguiculata* leaves are shown in Fig. 1. A slight increase in SOD activity was seen at 0.1 ppm SO_2 exposure. The SOD activity was found to be decreased slightly in leaves treated 0.2 ppm SO_2. It is clear from the experimental results that, fumigation with 0.1 ppm of SO_2 showed a slight increase in SOD, may be partly due to an increased metabolic activity or an increased SOD biosynthase.

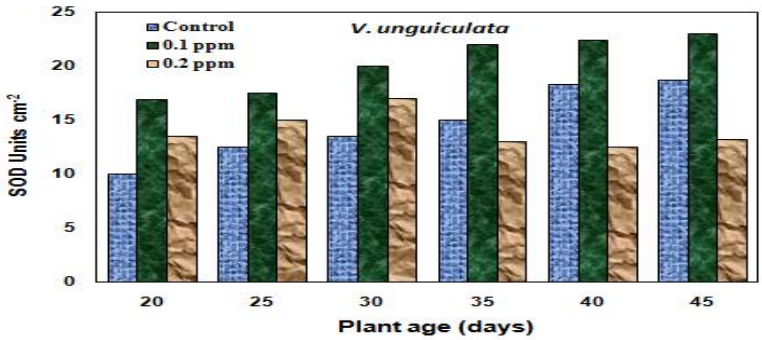

Fig. 1 Changes in SOD activity at different concentrations of SO_2 fumigated Vigna unguiculata

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B. SOD Activity at different Stages of Leaf Development

A plant response to stress is a complex phenomenon that appears to involve the synthesis of polyamines and a new set of proteins whose function is largely unknown. SOD can protect plant tissues from the superoxide radicals [13]. In this study, SOD activity was found to be significantly increased by SO_2 exposure when compared to normal control leaves (Table 1). The SOD activity was found to be in the order: mature leaves (198 mg/protein) > 50 – 70 % expanded leaves (209 mg/protein) > young leaves (216 mg/protein). Visible injury was observed in the following order: mature leaves > 50 – 70 % expanded leaves > young leaves. Greater SOD levels in young leaves compared to older leaves were associated with lower SO_2 sensitivities in these tissues. Hence, mechanism that reduce free radicals play an important secondary role in stress tolerance [14]

TABLE 1
SOD ACTIVITY IN LEAVES AT DIFFERENT STAGES OF DEVELOPMENT

Stage of Development	SOD activity (mg/protein)		Percentage of difference
	Control	SO_2 treated	
Younger Leaves (Less than 40 % expanded)	205	216	11 %
Expanded Leaves (50-70 % expanded)	170	209	39 %
Mature Leaves (Fully Expanded)	142	198	56 %

One unit of SOD activity is the amount of enzyme, which inhibited 50% of the cytochrome C reduction reaction at 25 °C.

C. Leaf injury And Enzyme Activities

An oxidative chain reaction of sulphite initiated by the superoxide ion produced in the Mehler reaction has been implicated in the damage of plants exposed to SO_2 [15]. Relationship between SOD activity and leaf damage by SO_2 were plotted as a function of leaf development [Fig. 2]. Leaves ranging from 70 – 95 % of their fully expanded leaves were more sensitive to SO_2 fumigation. Expanded leaves (50 – 70%) exhibited less injury. SO_2 did not injure very young trifoliate leaves of treated plants. SOD inhibits the chain oxidation of sulphite by scavenging O_2, thus SOD is one possible tolerance mechanism of leaf cells against SO_2 [16].

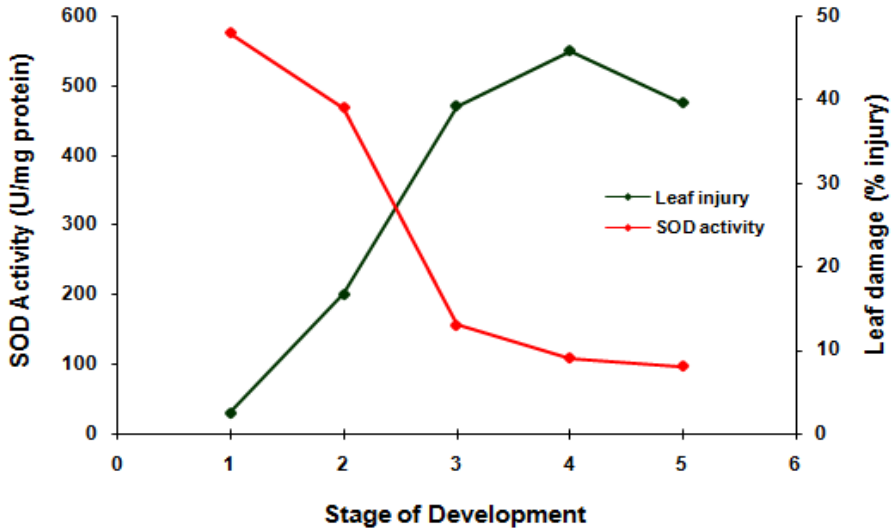

Fig. 2 Relationship between SOD activity and leaf development. Visible injury after (20 days after exposure to 0.1 ppm of SO_2 for 8 h) is expressed as % of leaf development. 1. Younger leaves 2. 40 – 50 % expanded leaves 3. 60 – 70 % expanded leaves 4. Fully expanded mature leaves 5. Primary leaves

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IV. CONCLUSIONS

In conclusion, SO_2 fumigation at different concentrations (0.1 and 0.2 ppm) showed increase in SOD activity in *V. uniguiculata* leaves when compared to untreated control leaves. Leaf injury was more in mature leaves than in younger leaves. SOD activity was found to be more in 0.1 ppm when compared to 0.2 ppm of SO_2 fumigation. On the whole, the data presented seem to prove that exposure to SO_2 can induce oxidative stress in *V. uniguiculata* leaves but the plant can scavenge those free radicals with the help of SOD and other antioxidant subatances.

V. ACKNOWLEDGMENT

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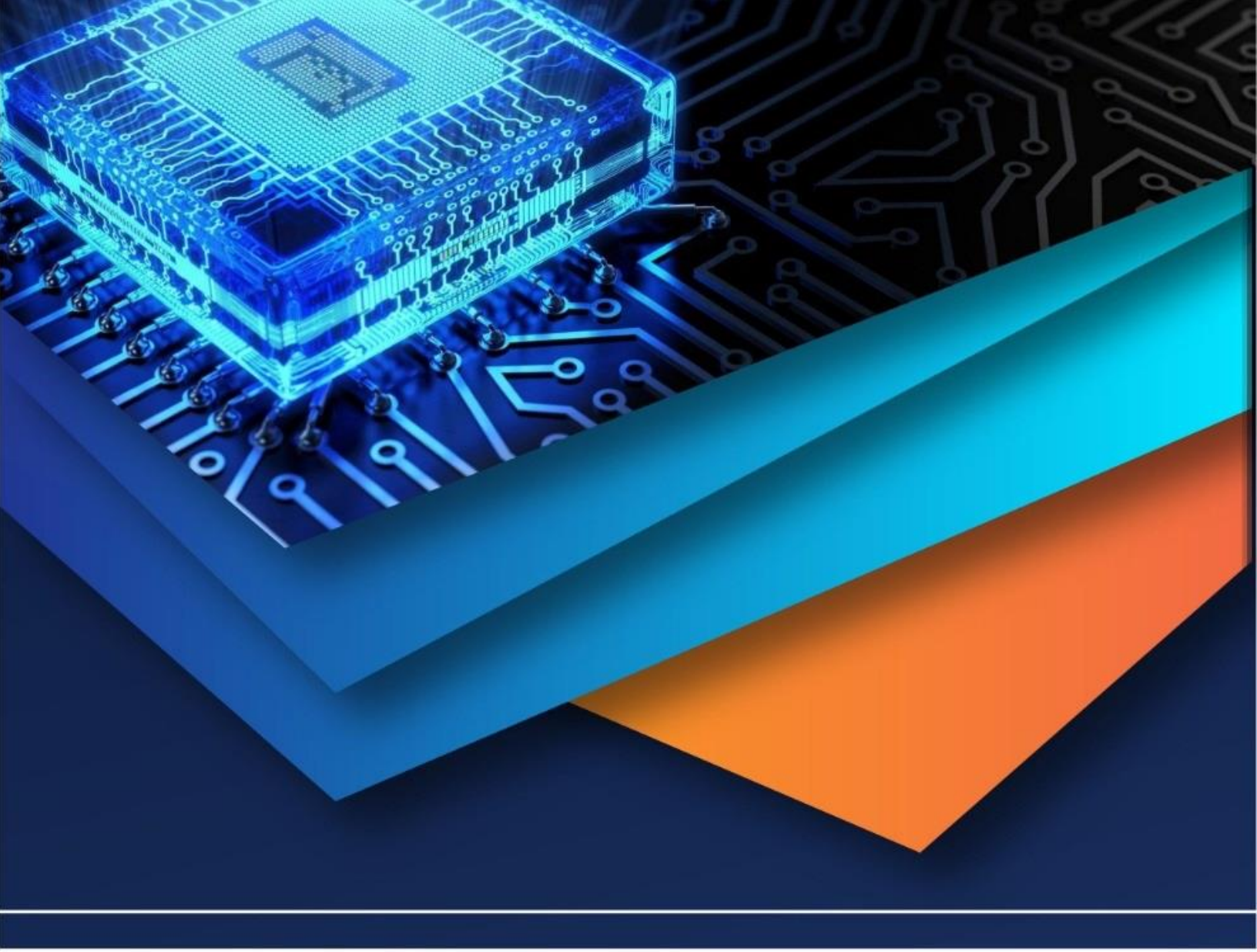

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