



iJRASET

International Journal For Research in
Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 6 Issue: IV Month of publication: April 2018

DOI: <http://doi.org/10.22214/ijraset.2018.4738>

www.ijraset.com

Call:  08813907089

E-mail ID: ijraset@gmail.com

Studies on Superoxide Dismutase Activity under SO₂ Fumigation in Vigna Unguiculata Leaves

Packialakshmi Balakrishnan¹

¹Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore - 641029, Tamilnadu, India

Abstract: The aim of the present study was to investigate the effect of sulphur dioxide (SO₂) on superoxide dismutase (SOD) activity at different stages of leaf development in Vigna unguiculata (V. unguiculata) leaves. V. unguiculata plants were fumigated with SO₂ 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 SO₂. 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 SO₂ fumigation. In conclusion, V. unguiculata plant tissues has its own defense mechanism and scavenging efficacy against toxic free radicals.

Keywords: Free radicals, Scavenging enzyme, SO₂, 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 be the main cause of air pollution. The phenomenon is particularly associated with ecologically unplanned industrialization and uncontrolled urbanization [1].

SO₂ is one of the major air pollutants, which causes serious damage to vegetation. SO₂ is emitted into the atmosphere by combustion of sulphur containing mineral ores [2]. The most important phytotoxic air contaminants are generally gaseous in nature. SO₂ 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 to the toxic effects of air pollutants [3]. When plants grow in an environment polluted with SO₂, it gets entry into the leaf tissue through stomata and subsequently produces H⁺, HSO₃⁻, and SO₃²⁻ 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 HSO₃⁻ and SO₃²⁻ get photooxidized to the less toxic SO₄²⁻ in chloroplasts, along with the production of O₂⁻ (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 causes oxidative damage to plants [6].

SOD has been examined in photosynthetic organisms [7]. It is an enzyme responsible for the breakdown of O₂⁻. It is a metalloprotein which catalyzes the dismutation of O₂⁻ to O₂ and H₂O₂ by altering the concentration of O₂⁻, SOD helps to prevent both direct toxicity from O₂⁻ and secondary toxicity from H₂O₂ [8]. SOD inhibits the chain oxidation of sulphite by scavenging O₂, thus it is one possible tolerance mechanism of leaf cells against SO₂ [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 SO₂ 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, Madurai 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 °C (day) and 28 °C (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.

B. Determination of LD₅₀

Plants were fumigated with various concentrations of SO₂ ranging from 0.1 – 1.0 ppm of SO₂. A 50% reduction in growth was observed at 0.25 ppm of SO₂. Therefore 0.1 ppm and 0.2 ppm concentrations were taken as experimental treatments for further studies. The SO₂ fumigation (2h/day) was started from 16th day of plant age and continued till 45 days of plant age (20 days of SO₂ 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 SO₂ 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 m³/min. The gas was uniformly distributed in the fumigation chamber through a network of perforated alkaline pipes arranged at the base. SO₂ 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 stand 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 SO₂ was calculated from a standard curve prepared by using standard solution of sodium sulphite in sodium tetra chloro mercurate (II). Each µg of SO₂ represented 0.1 ppm of SO₂ 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 pyrrolidone (PVP) and 2 g acetone powder. The suspensions were centrifuged at 13,000 rpm for 15 min at 4 °C. 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. SO₂ 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 SO₂ fumigation in *V. unguiculata* leaves are shown in Fig. 1. A slight increase in SOD activity was seen at 0.1 ppm SO₂ exposure. The SOD activity was found to be decreased slightly in leaves treated 0.2 ppm SO₂. It is clear from the experimental results that, fumigation with 0.1 ppm of SO₂ showed a slight increase in SOD, may be partly due to an increased metabolic activity or an increased SOD biosynthesis.

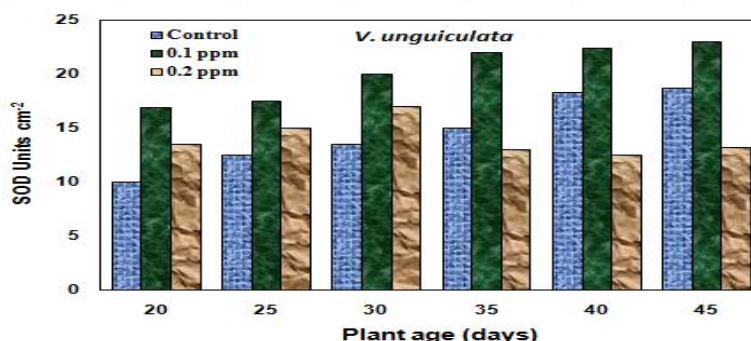


Fig. 1 Changes in SOD activity at different concentrations of SO₂ fumigated *Vigna unguiculata*

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 SO₂ 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 SO₂ 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 SO₂ [15]. Relationship between SOD activity and leaf damage by SO₂ were plotted as a function of leaf development [Fig. 2]. Leaves ranging from 70 – 95 % of their fully expanded leaves were more sensitive to SO₂ fumigation. Expanded leaves (50 – 70%) exhibited less injury. SO₂ did not injure very young trifoliolate leaves of treated plants. SOD inhibits the chain oxidation of sulphite by scavenging O₂, thus SOD is one possible tolerance mechanism of leaf cells against SO₂ [16].

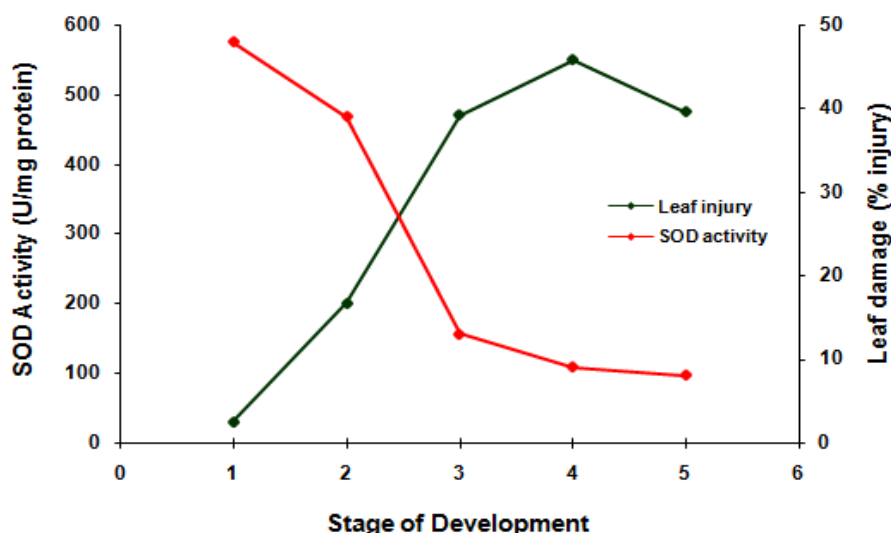


Fig. 2 Relationship between SOD activity and leaf development. Visible injury after (20 days after exposure to 0.1 ppm of SO₂ for 8 h) is expressed as % of leaf development. 1. Younger leaves 2. 40 – 50 % expanded leaves 3. 60 – 70 % expanded leaves 4. Fully expanded mature leaves 5. Primary leaves

IV. CONCLUSIONS

In conclusion, SO₂ fumigation at different concentrations (0.1 and 0.2 ppm) showed increase in SOD activity in *V. unguiculata* 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 SO₂ fumigation. On the whole, the data presented seem to prove that exposure to SO₂ can induce oxidative stress in *V. unguiculata* leaves but the plant can scavenge those free radicals with the help of SOD and other antioxidant substances.

V. ACKNOWLEDGMENT

The author gratefully acknowledges Dr. G. Renuga, Former Scientist, Department of Biochemistry, School of Biological Sciences, Madurai Kamaraj University, Madurai, India for providing laboratory facilities, guidance and encouragement.

REFERENCES

- [1] A. Calatayud, E. Barreno, "Chlorophyll a fluorescence, antioxidant enzymes and lipid peroxidation in tomato in response to ozone and benomyl," Environ Pollut., vol. 115, pp. 283-289, Dec. 2001.
- [2] S.S. Malhotra, D. Hocking, "Biochemical and cytological effects of sulphur dioxide on plant metabolism," New Phytol., vol. 76, pp. 227-237, Mar. 1976.
- [3] A.R. Wellburn, O. Majernik, F.A.M. Wellburn, "Effects of SO₂ and NO₂ polluted air on the ultrastructure of chloroplasts," Environ Pollut., vol. 3, pp. 37-49, Jan. 1972.
- [4] K. Tanaka, K. Sugahara, "Role of superoxide dismutase in defence against SO₂ toxicity and an increase in superoxide dismutase activity with SO₂ fumigation," Plant Cell Physiol., vol. 21, pp. 601-611, Jun. 1980.
- [5] J.N. Pandey, D.N. Rao, "Studies of coal smoke sulphurdioxide pollution on wheat-I Exposure technique and correlation of morphological effects with sulphurdioxide dose," Indian J Air pollut Cont., vol. 1, pp. 9, 1978.
- [6] D.W. Cowling, A.W. Bristow, "Effects of SO₂ on sulphur and nitrogen fractions and on free amino acids in perennial ryegrass," J Sci Food Agri., vol. 30, pp. 354-360, Apr. 1979.
- [7] J. Lumsden, R. Cammack, D.O. Hall, "Superoxide dismutase in photosynthetic organisms provides an evolutionary hypothesis," Nature., vol. 257, pp. 670-672, Oct. 1975.
- [8] R. Nageswara R, Madamanchi, R.G. Alscher, "Metabolic Bases for Differences in Sensitivity of Two Pea Cultivars to Sulfur Dioxide," Plant Physiol., vol. 97, pp. 88-93, Sep. 1991.
- [9] K. Asada, "Formation and scavenging of superoxide in chloroplasts, with relation to injury by sulfur dioxide," Natl Inst Environ Stud., Res Rep. 11, pp. 165-179, 1980.
- [10] P.W. West, G.C. Gaeke, "Fixation of Sulfur Dioxide as Disulfidomercurate (II) and Subsequent Colorimetric Estimation," Anal Chem., vol. 28, pp. 1816-1819, Dec. 1956.
- [11] J.M. McCord, I. Fridovich, "Superoxide dismutase. An enzymic function for erythrocyte hemocuprein," J Biol Chem., vol. 244, pp. 6049-6055, Nov. 1969.
- [12] C.H. Foyer, P. Descourvières, K.J. Kunert, "Protection against oxygen radicals: an important defence mechanism studied in transgenic plants," Plant Cell Environ., vol. 17, pp. 507-523, May. 1994.
- [13] J.M. Tepperman, P. Dunsmuir, "Transformed plants with elevated levels of chloroplastic SOD are not more resistant to superoxide toxicity," Plant Mol Biol., vol. 1, pp. 501-511, Apr. 1990.
- [14] C. Bowler, W. Van Camp, M. Van Montagu, D. Inze, "Superoxide dismutase in plants," Crit Rev Plant Sci., vol. 13, pp. 199-218, 1994.
- [15] L. Tang, S.Y. Kwon, S.H. Kim, J.S. Kim, J.S. Choi, K.Y. Cho, C.K. Sung, S.S. Kwak, H.S. Lee, "Enhanced tolerance of transgenic potato plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against oxidative stress and high temperature," Plant Cell Rep., vol. 25, pp. 1380-1386, Dec. 2006.
- [16] D. Grill, D. Esterbauer, K. Hellig, "Further studies on the effect of SO₂ pollution on the sulfhydryl systems of plants," Phytopathol Z., vol. 104, pp. 264-271, 1982.



10.22214/IJRASET



45.98



IMPACT FACTOR:
7.129



IMPACT FACTOR:
7.429



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Call : 08813907089  (24*7 Support on Whatsapp)