Proc. Indian natn. Sci. Acad.
Vol. 43, Part B, No. 6, pp. 236-241 (1977)
Ecology

STUDY OF SOME FACTORS IN PLANTS CONTROLLING THEIR SUSCEPTIBILITY TO SO_2 POLLUTION*

by C. S. CHOUDHARY *and* D. N. RAO, *Department of Botany, Banaras Hindu University, Varanasi* 221005

(Communicated by Professor R. Misra, FNA)

(Received 12 *December,* 1977*)*

INTRODUCTION

In recent years, much investigations have been conducted on phytotoxic effects of sulphur dioxide (Thomas *et al.*, 1950; Daines, 1968 Jacobson & Hill, 1971 and Mudd, 1975). A proper understanding of phytotoxicity and metabolic inhibition by SO_2 has remained a difficult problem due to the pollutant dual properties of oxidation and reduction.

Bleasdale (1952) reported that the toxicity of SO_2 was due to its reducing property which upsets the equilibrium between sulphydryl ions and more oxidized sulphite ions by increasing the accumulation of former in the plant body. He further suggested that the plants tolerant to SO_2 are perhaps able to control the accumulation of sulphydryl group in their body within safe limits. Choudhary & Rao (1977) reported that less number of stomata, high water retention percentage and chlorophyll content of leaves seem to reduce the sensitivity of plants to SO_2 pollution. Ballantyne (1973) suggested that the variations in susceptibility of plants to SO_2 were due to differences in sulphite inhibition of phosphorylating activity and when the ratio of oxidized to reduced sulphydryl compound increased in the presence of SO_2 fumigation, extensive foliar damage occurred. Harrer & King (1941) studied the function of ascorbic acid in plants and reported that it protects the sulphydryl group from oxidation at *p*H value above 7.6; but at low *p*H values, ascorbic acid gets oxidized into dehydroascorbic acid which is reversible in nature.

In the present study, an attempt has been made to understand the basis of plants' susceptibility to SO_2 pollution with special reference to ascorbic acid content and cell-sap *p*H.

MATERIALS AND METHODS

Fumigation

Two plants of known susceptibility to SO_2 were selected. Of these, one was maize (*Zea mays*) and the other soyabean (*Glycine max*) known to be resistant and

* This paper has been presented in the IV Annual Meeting of the Society for Advancement of Botany.

sensitive, respectively, to SO_2. Seeds of these plants were sown in two separate beds and when the plants had attained the age of 30 days, they were fumigated with a known concentration of SO_2, enclosed within a m^3 plastic chamber. The fumigation continued daily for 2 hours with 1.0 ppm SO_2 between 30 and 40 days and subsequently with 0.7 ppm between 50 and 80 days of their ages. In between the age of 40 to 50 days, the fumigation was suspended and the plants were allowed to recover from acute injury which they had undergone during the fumigation with 1.0 ppm SO_2 (Table I).

TABLE I

Fumigation and sampling schedule of maize and soyabean plants (Fumigation started when the plants attained the age of 30 *days)*

Age (days)	Experimental condition	Cumulative SO_2 dose ppm Concn. × hours × days	Sampling day
1-30	Normal	—	30th
30-40	Fumigation	$1 \times 2 \times 10 = 20$	40th
40-50	Recovery	—	50th
50-60	Fumigation	$0.7 \times 2 \times 10 = 14+20=34$	60th
60-70	Fumigation	$0.7 \times 2 \times 20 = 28+20=48$	70th
70-80	Fumigation	$0.7 \times 2 \times 30 = 42+20+62$	80th
80-90	Recovery	—	90th (Harvesting)

Analysis of plant samples

The plants were sampled at 30, 40, 50, 60, 70, 80 and 90 days intervals and cell-sap *p*H as well as ascorbic acid content of sampled leaves, were determined.

Cell-sap p*H*

For cell-sap *p*H determination, a 5 g sample of fresh leaves was homogenized and the *p*H of homogenate was measured with a photovolt electronic *p*H meter, using a glass electrode (Table II).

Ascorbic acid content

Preparation of standard solution : In one litre of double distilled water 20 mg of ascorbic acid were dissolved. A 10 ml aliquot of this solution was taken in a 50 ml conical flask to which 2 ml glacial acetic acid and 2.5 ml chloroform were added. The content in the conical flask was shaken well and titrated against 2, 6 dichlorophenol-indophenol dye solution. The titre value of the standard solution was noted when the solution in the conical flask attained a faint pink permanent colour.

TABLE II

Ascorbic acid content (mg per 100 gm fresh leaf) and cell-sap pH of Control (C) and Fumigated (F) Glycine max *and* Zea mays *plants*

Plant age in days	Cumulative SO_2 dose	Ascorbic acid content (mg per 100 gm fresh leaf)				Per cent loss of Ascorbic acid in fumigated plant with respect to control		Cell-sap *p*H			
	(l × t)	*Glycine max*		*Zea mays*		*Glycine max*	*Zea mays*	*Glycine max*		*Zea mays*	
—	—	C	F	C	F	—	—	C	F	C	F
30	—	65·38	—	146·71	—	—	—	6·30	—	6·50	—
40	20	72·35	40·25	156·25	104·74	44·36	32·94	6·35	5·50	6·45	5·90
50	Recovery	81·50	62·57	130·80	112·25	23·22	21·82	6·30	5·60	6·42	5·80
60	34	68·35	44·31	105·21	81·42	35·21	22·60	6·25	5·40	6·38	5·80
70	48	48·25	23·13	70·65	41·63	52·06	41·07	6·20	5·40	6·35	5·80
80	62	30·42	9·52	37·48	18·13	68·34	51·60	6·30	5·35	6·30	5·80
90	Recovery	14·80	5·81	19·12	9·21	60·70	51·83	6·20	5·35	6·25	5·60

Preparation of 2,6 dichlorophenol indophenol dye solution

In 500 ml hot water 20 mg of the dye 2, 6-dichlorophenol indophenol was dissolved. After cooling, the solution was diluted with double distilled water, the total volume was made up to 1000 ml and filtered to remove the undissolved dye. The filtrate was stored in a refrigerator for sometime and used as standard solution.

Preparation of test solution

A 5 g sample of fresh leaves was ground well in 50 ml distilled water and the water extract was filtered through a double-layer muslin cloth. Ascorbic acid being soluble in water was easily extracted from the leaf tissue in this manner.

To 2 ml of the extract was transferred in a 50 ml conical flask with the help of a pipette, 8 ml of distilled water, 2 ml of glacial acetic acid and 2.5 ml of chloroform were added. This reaction mixture was then titrated against the dye solution. The titre value was noted when solution in the flask attained a permanent faint pink colour.

The ascorbic acid content was determined by using the following formula given by Plumer (1971),

$$\text{Ascorbic acid of test solution (mg/100 ml)} = \frac{\text{T-Bl}}{\text{St-Bl}} \times 2 \times \text{dilution}$$

T = Titre value of test solution

St = Titre value of standard solution

Bl = Titre value of Blank solution

Results

At all ages of plants, the *p*H values of cell-sap of leaves of soyabean and maize were found to decrease after fumigation with SO_2 (Table II). In both plants there was a gradual decrease in cell-sap *p*H at higher cumulative SO_2 doses which are much more pronounced in soyabean than maize. The *p*H values of control soyabean and maize leaves were 6.3 and 6.5 respectively, which are reduced to 5.3 in former and 5.8 in the latter after 110 hours of SO_2 fumigation.

From the data presented in Table II, it is obvious that the control leaves of maize were richer in ascorbic acid which was twice as much as in those of soyabean; the maximum values of ascorbic acid being 156.25 mg/100 g fl of maize and 81.50 mg/100 g fl of soyabean. The ascorbic acid contents, however, gradually decreased at increasing dosage of SO_2 at all ages of plants. The decrease was more in soyabean than in maize, the maximum decrease being 68.35% in the former and 51.6% in the latter.

Discussion

Higher concentrations of SO_2 cause foliar damage in plants and affect their physiological processes (Thomas, 1961; Mudd, 1975). Barker & Mapson (1952, 1955) and Mapson (1958) reported that foliar damage in plants disturbed the balance between ascorbic and dehydroascorbic acids by reducing the ascorbic acid content. He further suggested that the cellular disorganization or a poisoning of specific enzyme decreased the amount of ascorbic acid to increase dehydroascorbic acid content.

Tanaka, *et al.* (1973) reported that exposure to SO_2 inactivated the photosynthetic enzyme ribulose 1, 5-diphosphate carboxylase by sulphonation of its —SH, a sulphydryl group; and glycolic acid oxidase by hydroxy sulphonate, an additional reaction product of aldehyde and SO_2 and reduced the photosynthesis. Therefore, it is possible that the decrease in amount of ascorbic acid in fumigated plants might be due to enzyme poisoning and sulphonation of their —SH group. Hallerman (1937) reported that the activity of enzymes depend upon the structural integrity of —SH group in molecules. The —SH group in molecules, present in glutathione and cystein were disrupted as a result of SO_2 fumigation and accumulation of HSO_3^- and $SO^{2-}{}_2$ ions, which imbalanced the equilibrium between sulphydryl group and incompletely oxidized sulphur compound (McMuller, 1960; Laughman, 1964). Ballantyne (1973) has shown that the exposure of SO_2 altered the ratio of oxidized to reduced sulphydryl compound and increased the foliar damage. It is suggested that ascorbic acid protects the —SH group in molecules from oxidation (Harrer & King, 1941). Therefore, it is assumed that the higher amount of ascorbic acid in cells provides greater protection to sulphydryl group, a functional integrity of protein molecules. From this it may be concluded that twice the amount of ascorbic acid in maize than in soyabean makes the former more resistant to SO_2 than the latter.

It may be summarized that the tolerance of plants to SO_2 is dependent on the degree of stability of sulphydryl compound in them (Bleasdale, 1952). Probably, the reduction of ascorbic acid in presence of SO_2 is due to its conversion to dehydro ascorbic acid or oxalic acid, and other convertible carbohydrate (Hogler & Herman, 1973; Yong & Loewus, 1975). The ascorbic acid activity is very much controlled by *p*H which is more at higher *p*H and less at lower values. Since sulphur dioxide lowered the *p*H values more in soyabean than in maize, consequently there was a greater decrease of ascorbic acid in the former than in the latter (Table II). The greater decreases of ascorbic acid content and cell-sap *p*H in soyabean leaves are perhaps responsible for its sensitivity to SO_2 pollution. In the converse manner, the smaller decrease of cell-sap *p*H and ascorbic acid in leaves of maize makes the plant resistant to SO_2 pollution (Table II).

On the basis of this study, it may be suggested that the cell-sap *p*H and ascorbic acid play an important role in determining the susceptibility of plants to SO_2 pollution. It appears that maize plant is resistant because of its high values of cell-sap *p*H and ascorbic acid content and likewise soyabean is sensitive because of its low values of cell-sap *p*H and ascorbic acid content.

Acknowledgement

The authors are thankful to the Head of the Botany Department, Banaras Hindu University, Varanasi, for providing them with laboratory facilities.

References

Bleasdale, J. K. A. (1952). Air pollution and plant growth, in "Smokeless air", report in *J. Park Admin.*, **18,** 300-301 (Hort. Abstr. 1954 No. 1084).

Ballantyne, D. (1973). Sulphite inhibition of ATP formation in plant mitochondria. *Phytochemistry*, **12,** 1207.

Barker, J & Mapson, L.W. (1952) *New phytologist*, **51,** 20-115.

———(1955). *Proc. Roy. Soc. Lond*, **B 143,** 523-549.

Choudhary, C. S. & Rao, D. N. (1977). Characterisation and Evaluation of plant susceptibility to SO_2 pollution. In: 4th National Convention of Environmental Engineering; B. H. U. Varanasi (in press).

Daines, R. H. (1968). Sulphur dioxide and plant response. *J. Occupat. Medic.*, **10,** 516-524.

Hallerman, L. (1937). *Physiol. Revs.* **17,** 454-484.

Hogler, L. & Herman R. H. (1973). Oxalate metabolism I. *Am. J. Clin. Nutr.*, **26,** 758-765.

Harrer, C. J. & King, C. G. (1941). *J. Biol. Chem.*, **138,** 111-121.

Jacobson, J. S. & Hill, A. C. eds. (1971). Recognition of Air Pollution Injury to vegetation. A Pictorial Atlas. Air Pollution Control Association, Pittsburg.

Laughman, B. C. (1964). Sulphur absorption and Metabolism in plants. *Agrochemica*, **8,** 189.

Mapson, L. W. (1958). Metabolism of Ascorbic acid in plants part I. Function. *A. Rev. Pl. Physiol.*, **9,** 119-150.

Mudd, J. B. (1975). Sulphurdioxide. In: *Responses of Plants to Air Pollution* (Ed. J. B. Mudd and T. T. Kozlowski) pp. 9-22. Academic Press, New York.

McMuller, A. I. (1960). Thiols of Low molecular weight in *Havea bransilliensis* latex. Acta *Biochem. Biophys.* **41,** 152.

Plumer, D. T. (1971). *An Introduction to Practical Biochemistry*, Published by McGraw Hill Book Company (U. K.) Limited Maiden Head Berkshire England, pp. 223-230.

Tanaka, H., Takanashi, T. & Yatazawa, M. (1973). Experimental studies on SO_2 injury in higher plants. III Inhibitory effect of sulphite ion on $^{14}CO_2$ fixation. *Water, Air and Soil Pollution*, **3,** 1974, 11-16.

Thomas, M. D., Hendricks, R. H. & Hill, G. R. (1950). The sulphurmetabolism in plants. Effect of sulphur dioxide on vegetation. *Ind. Eng. Chem.* **42,** 2231-2235.

Thomas, M. D. (1961). Effects of air pollution on plants. In: *Air Pollution*. WHO. Geneva Monog. Ser, **46,** pp. 233-278.

Young, J. C. and Loewus, F. A. (1975). Metabolic conversion of L-Ascorbic acid to oxalic acid in oxalate accumulating plants. *Pl. Physiol.* **56,** 283-285.