MITIGATING EFFECTS OF EXOGENOUSLY SUPPLIED ASCORBIC ACID ON *VIGNA CATJANG* WALP. GERMINATING SEEDS EXPOSED TO SULPHUR DIOXIDE

N. S. R. KRISHNAYYA and S. J. BEDI
Department of Botany, Faculty of Science, The M. S. University of Baroda, Baroda 390 002, India.

THE adverse effects of SO_2 on plants have received a lot of attention[1,2]. Differences have been reported in metabolic responses of plants with SO_2 fumigation[3]. Increase in foliar sulphur content was reported[4] in plants exposed to SO_2. In ozone-resistant cultivars, higher ascorbic acid content was reported[5]. Higher amount of ascorbic acid occurs in all growing parts of plants. It is primarily a powerful reductant of protochlorophyllide[6]. The ability to reduce disulphides and to reduce or combine with thiol group oxidants is exemplified by the ascorbic acid system[7]. Recently it was reported that species with a high ascorbic acid content are relatively SO_2-resistant[8]. This note reports the effect of exogenously supplied ascorbic acid in mitigating the adverse effects of SO_2 on seed germination and storage protein mobilization.

Certified *Vigna catjang* Walp. seeds (var. pusa) were employed in this investigation. One set was soaked in water and the other in solution containing 150 μg/ml ascorbic acid for 8 h. Later they were transferred into six sets of petri plates, each containing 4 pairs of plates (10 seeds in each petri plate). Of the 6 sets, 3 were not treated with ascorbic acid and the other 3 sets were treated with 150 μg/ml ascorbic acid solution. Two sets, one each from treated and untreated were taken as control, two sets were exposed to 0.5 ppm SO_2 for 1 h and the third pair was exposed to 0.5 ppm SO_2 for 2 h. The required concentration of SO_2 was obtained from a SO_2 cylinder by diluting with air and the concentration of SO_2 was monitored[9] throughout the experimentation. During exposure the temperature inside the chamber was 29°C and the room temperature was 26 ± 2°C. Three exposures at a regular interval of 24 h were given. The percentage germination and the protein content (using Folin phenol reagent[10]) in the cotyledons were determined after 24 h and 48 h. The protease enzyme activity was estimated at 48 h[11]. The tissue was ground in 0.01 M tris buffer (pH 8) and 0.5 ml of the homogenate was added to 2% heat-denatured casein. The tubes were kept in a water bath at 37°C for 1 h and the reaction was terminated by adding 1 ml of 20% trichloroacetic acid. From the supernatant, suitable aliquots were taken and the amount of tyrosine liberated was estimated by Lowry's method. The enzyme activity was estimated by measuring the number of micromoles of tyrosine liberated/h/g. fresh wt. After the third exposure, 100 mg of embryonal axis was taken and ground in 8 ml mixture of chilled 5% metaphosphoric and 10% acetic acid solution. The ascorbic acid was estimated by using dinitro phenyl hydrazine as the colour developing reagent[12]. After the third exposure the seedlings were oven-dried at 60°C for 48 h, powdered and passed through a 100 μ sieve. Sulphur was estimated by using $BaCl_2$-tween 80 as the turbidometric reagent[13].

The results are given in table 1. The reduction in germination was greater in untreated-exposed as compared to treated-exposed sets. At 24 h the

Table 1 *Mitigating effects of exogenously supplied ascorbic acid on Vigna germinating seeds exposed to SO_2*

Parameters		P_0	P_1	P_2
Per cent germination after 24 h	UTR	70.0	57.5	50.0
	TR	72.5	67.5	60.0
Per cent germination after 48 h	UTR	77.5	60.0	52.5
	TR	82.5	75.0	62.5
Total proteins after 24 h (mg/g. fr. wt.)	UTR	134.2 (0 5)	161.9 (0.47)	167.3 (0.22)
	TR	128.9 (0.4)	150 0 (0.44)	153.9 (0 49)
Total proteins after 48 h (mg/g. fr. wt.)	UTR	74.2 (0.29)	90.3 (0 60)	105.2 (0.12)
	TR	73 6 (0.54)	76.9 (0.29)	85.4 (0.3)
Protease activity (μmol of tyrosine liberated/h/g. fr. wt)	UTR	21 8 (0.14)	11 5 (0.36)	7.7 (0 6)
	TR	23.4 (0.45)	16.3 (0.7)	11.05 (0.9)
Ascorbic acid (μg/g. fr. wt.)	UTR	.358 0 (0.79)	303 0 (0.86)	278 0 (0.75)
	TR	372.0 (0.3)	343 0 (0 3)	321.0 (0.65)
Sulphur content (mg/g. dry wt.)	UTR	6 07 (0 04)	6 43 (0 04)	6.64 (0.04)
	TR	6.1 (0.03)	6.21 (0 03)	6 32 (0 02)

P_0, Control; P_1, exposed to 0.5 ppm SO_2 for 1 h; P_2, exposed to 0.5 ppm for 2 h; UTR, Untreated; TR, Treated with ascorbic acid; All the readings are an average of 4 observations; Values in parentheses represent ± standard deviations.

reduction in germination for 1 and 2 h was 40 and 50% in untreated-exposed while in treated-exposed it was 30 and 40%. After 48 h there was a slight increase in seed germination of untreated-exposed and the reduction was 40 and 48% whereas in treated-exposed sets it was 25 and 38%. The protease enzyme activity which hydrolyses storage protein and helps in seedling growth, was less in untreated-exposed sets as compared to treated-exposed sets. The above findings show that ascorbic acid mitigates the adverse effects of SO_2 on seed germination and on protease activity. The protein content in cotyledons of exposed sets was higher, showing that hydrolysis was reduced due to SO_2. As the exposure period increased the negative effects of the SO_2 increased (table 1). The ascorbic acid content was greater in treated sets indicating that it has been taken directly by the seedling and therefore has an important role in plant metabolic pathways and acts as a reductant[7]. Sulphur dioxide accumulates in plants as SO_3^{2-} and ferredoxin reductase system is required for the photoreduction to sulphide[14]. The excess of sulphur accumulated in plant as sulphate and sulphite which causes injury is volatilized from the leaves as H_2S and this reaction is light-dependent. Light is needed for the supply of reductant[15]. Ascorbic acid acts as a powerful reductant and seems to mediate the reduction of sulphite to H_2S thereby reducing the toxicity of SO_2[8]. In the present study the sulphite accumulated in the treated-exposed seeds might have converted into H_2S using ascorbic acid as a reductant thereby reducing the toxicity of SO_2. The lower increase in the seedling sulphur content of treated-exposed sets supports this assumption. Further investigation is necessary to understand the resistance of plants against air pollution.

One of the authors (NSRK) is grateful to UGC, New Delhi, for financial assistance.

3 July 1987; Revised 30 March 1988

1. Bell, N. B. and Clough, W. S., *Nature (London)*, 1973, **241,** 49.
2. Malhotra, S. S. and Hocking, D., *New Phytol.*, 1976, **76,** 227.
3. Jager, H. J., Bender, J. and Grunhage, L., *Environ. Pollut.*, 1985, **A39,** 317.
4. Shanklin, J. and Kozlowski, T. T., *Environ. Pollut.*, 1984, **A36,** 311.
5. Lee Edward, H., Jersey James, A., Carol Gifford and Jessee Bennett, *Environ. Exp. Bot.*, 1984, **24,** 331.
6. Rudolph, E. and Bukatsch, F., *Planta Jena*, 1966, **69,** 124.
7. Mapson, L. W., In: *Vitamins and hormones: Advances in research and applications*, (eds) R. S. Harris, G. F. Marrian and K. U. Thimann, Academic Press, New York, 1953, Vol. 11, p. 1.
8. Varshney, S. R. K. and Varshney, C. K., *Environ. Pollut.*, 1984, **A35,** 285.
9. West, P. W. and Gaeke, G. C., *Anal. Chem.*, 1956, **28,** 1916.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193,** 265.
11. Tappel, A. L., *Anal. Biochem.*, 1968, **23,** 466.
12. Joseph, R. H., *Methods Biochem. Anal.*, 1954, **1,** 115.
13. Garrido, M. L., *Analyst*, 1864, **89,** 61.
14. Tamura, G. and Itoh, S., *Agric. Biol. Chem.*, 1974, **38,** 225.
15. Silvius, E. J., Baer, C. H., Sherman Dodrill and Homer Patrick, *Plant Physiol.*, 1976, **57,** 799.

CHARACTERIZATION OF A STREPTOMYCIN-RESISTANT MUTANT OF *SCENEDESMUS DIMORPHUS* (TURP.) KUETZ

VEENA BAJAJ and PUSHPA SRIVASTAVA
Department of Botany, University of Rajasthan, Jaipur 302 004, India.

STREPTOMYCIN has been used in algae mainly to study its effect on chlorophyll formation and in isolation of apochlorotic races of *Euglena*[1,2]. Ebringer[3,4] attributed inhibition of chlorophyll synthesis to the chemical structure of streptomycin in which the carbohydrate molecule contains glycolate linkages and a large number of hydrophilic groups (amino or guanidine groups)[5].

A few reports[6–10] are on record, pertaining to the effect of streptomycin on pigment production in algae. The present study incorporates biochemical analysis of chlorophyll pigments quantitatively and the qualitative analysis of free and protein bound amino acids of streptomycin-resistant mutant of *S. dimorphus*.

A wide range of concentrations 0.05 to 2 mg of streptomycin per 100 ml of the medium were used but 1 mg/100 ml proved lethal[11]. Cultures from sub-lethal concentration (0.5 mg/100 ml) were sub-cultured several times in fresh nutritive medium and used for analysis. Chlorophyll pigments were