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EFFECT OF AQUEOUS SO_2 INCUBATION ON STOMATAL BEHAVIOUR OF THE LEAF EPIDERMAL STRIPS OF *CAJANUS CAJAN* (L.) MILLSPAUGH AND *AMARANTHUS PANICULATUS* (L.)

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Abstract

The widespread use of coal and petroleum as energy sources for industries has led to the emission of large quantities of sulphur dioxide (SO_2) into the atmosphere. Sulphur dioxide is a gaseous pollutant widely diffused in the world today. The stomatal behaviour of *Cajanus cajan* and *Amaranthus paniculatus* under aqueous SO_2 exposure were studied in isolated epidermal strips of their abaxial leaf surfaces. After exposed to illumination for 1 h, the strips were transferred to small petri dishes (5 cm) containing 0, 10, 20, 30, 40, 50, 100 and 250 ppm aqueous SO_2 solutions. Stomatal aperture size was measured at regular time intervals under a compound microscope using precalibrated occular micrometer. The lower aqueous SO_2 concentrations initially increased the stomatal opening, followed by a closure with increasing duration of SO_2 exposure that was responsible for the stomatal opening differs in pigeonpea and amaranth. The distribution of potassium in the guard cells was detected by histochemical technique. In pigeonpea, aqueous SO_2 indicated that the guard cells of control and 10 ppm SO_2 treated epidermal strips exhibited higher quantities of potassium accumulation than the guard cells exposed to higher concentrations of SO_2. On the other hand, in amaranth the guard cells of control epidermal strips showed higher potassium levels than SO_2 treated ones at 2 h of incubation. Further the data indicated that amaranth appears to be more sensitive to SO_2 than pigeonpea.

Keywords: *Amaranthus*, *Cajanus cajan*, guard cells, leaf, stomatal behaviour, SO_2.

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Introduction

Air pollution receives one of the prime concerns in India. Presently, SO_2 is the main issue pertaining to air pollution problems in developing countries, where it contributes both to urban pollution and to regional acid depositions (Cofala *et al*., 2004). Sulphur dioxide is a prime pollutant which is released directly to the atmosphere from domestic and industrial processes, particularly those using petroleum and coal combustion (Wellburn, 1998; Emberson *et al*., 2001; Gurjar *et al*., 2004). SO_2 can be oxidized in the atmosphere to form sulphate aerosols that contribute to acid deposition (Holleman, 2001). Thus elevated level of sulphate ions (SO_4^{2-}) concentrations in rain water are due to strong SO_2 emissions from coal fired thermal power plants (Demirak, 2007). However, it is very toxic at high concentration, so attention has been paid to the phenomenon of destroyed enzyme function and photosynthesis by SO_2, which inhibits plant growth and development and thereby markedly decreases crop yield (Bressan *et al*., 1978; Darrall,1989).

The exchange of gases between the atmosphere and a cellular system is a free diffusion process. Aqueous sulphur dioxide contains non-ionic forms of dissolved sulphur dioxide (SO_2 x H_2O), sulphurous acid (H_2SO_3) and the ionic forms of sodium bisulphite (HSO_3^-) and sulphite (SO_3^{2-}). Diffusion of gases into a leaf is hindered by several barriers. Stomata can regulate entry of a gas into the interior of a leaf. The different forms of SO_2 formed in aqueous medium enter the leaf through the open stomata, cell walls and cell membranes interfere with its normal functioning (Nieboer *et al*., 1976; Nieboer *et al*., 1977). Sulphur dioxide penetrates plants mainly through stomata and to a considerable extent through the epidermal surface of leaves (Garsed, 1981). Within the leaves, SO_2 is converted to products of reactivity which affect leaf metabolism. Hence, it is considered of great value to study the effect of aqueous SO_2 on the stomatal behaviour of isolated epidermal strips of *Cajanus cajan* and *Amaranthus paniculatus*.

MATERIALS AND METHODS

Seeds of pigeonpea (*Cajanus cajan* (L.) Millsp. cv.PDM1), an important pulse crop and amaranth (*Amaranthus paniculatus* L. a local cultivar), popular green leafy vegetable consumed all over India were selected for present investigation. The fully expanded third leaves were selected from the top of pigeonpea and amaranth plants grown separately for one month in pots for the study. Epidermal strips were separated from either side of the main veins of lower epidermis and floated on distilled water until sufficient material has been collected. To avoid the cell injury and mesophyll contamination, the strips were peeled at right angles to the lamina (Willmer and Mansfield, 1969; Weyers and Travis, 1981). The epidermal strips were carefully screened by microscopic examination to eliminate further mesophyll contamination if any. The strips were kept at room temperature of 28 ±1ºC in dark for 1 h to obtain consistent stomatal responses to illumination and then the strips were exposed to illumination (195µmol $m^{-2}s^{-1}$). After 1 h exposure the strips were transferred to small petri dishes (5 cm) containing 0, 10, 20, 30, 40, 50, 100 and 250 ppm aqueous SO_2 solutions. Stomatal aperture size was measured at regular time intervals under a compound microscope using precalibrated occular micrometer.

Preparation of aqueous sulphur dioxide

Sulphur dioxide was prepared in the laboratory by reacting sodium metabisulphite with concentrated H_2SO_4 and the generated gas was collected into distilled water. Aqueous SO_2 concentration was determined titrimetrically according to the method of Vogel (1961). Fresh stock solution of 1000 ppm concentration was prepared and from it the various concentrations of SO_2 were prepared by diluting with distilled water. The pH was adjusted to 6.9 by adding dilute NaOH. It was reported that 1 ppm SO_2 in air gives 1000 ppm in aqueous solution (Puckett *et al.*, 1973; Saunders and Wood, 1973; Malhotra, 1977).

Potassium fluxes during stomatal opening and closure

Potassium ions in the guard cells were detected histochemically according to the method of Fischer (1968) as modified by Rao and Anderson (1983). The epidermal strips were incubated for 2 h in the different concentrations of aqueous SO_2. The strips were then removed and immersed in sodium cobaltinitrite stain for 30 min. All the above steps were carried out in an ice bath. The strips were then removed and were floated on distilled water. Later they were placed in a mixture of 1:1 ammonium sulphide and 50% glycerine for 1 min, washed in distilled water twice and mounted on a glass slide using glycerine. The epidermal strips were observed microscopically for the degree of extent to which the black cobaltous sulphide precipitate formed within the guard cells and were scored as plus numbers. The highest dense appearance as maximum plus numbers and the minimum as single plus.

RESULTS AND DISCUSSION

Changes in stomatal aperture were determined using the incubation of detached isolated epidermal strips of lower (abaxial) epidermis of both pigeonpea and amaranth in different aqueous SO_2 concentrations.

The stomatal aperture size of pigeonpea showed certain initial increase at 10, 20 and 30 ppm aqueous SO_2 followed by a decline with increasing duration of exposure. All the other higher SO_2 concentrations resulted in a continuous decrease of stomatal aperture size with increasing duration of exposure. The aperture size was affected maximum at 250 ppm aqueous SO_2. On the other hand, the stomatal aperture increased continuously with increasing duration of exposure in the control epidermal strips (Figure-1a; Plate-1).

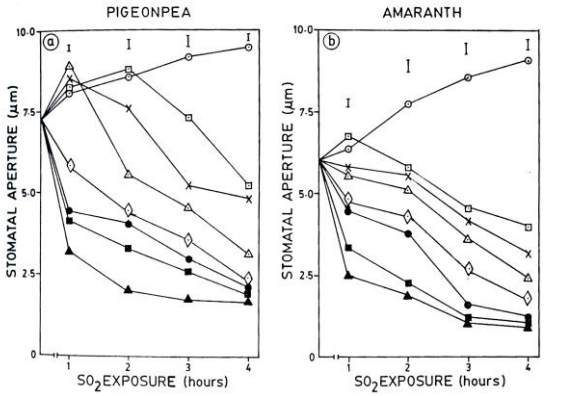

Figure-1: The effect of aqueous SO_2 on the stomatal aperture of pigeonpea (a) and amaranth (b) (vertical lines represent S.E.) ○- 0 ppm; □-10 ppm; ×-20 ppm; Δ-30 ppm; ◊- 40 ppm; ●-50 ppm; ■-100 ppm; ▲-250 ppm.

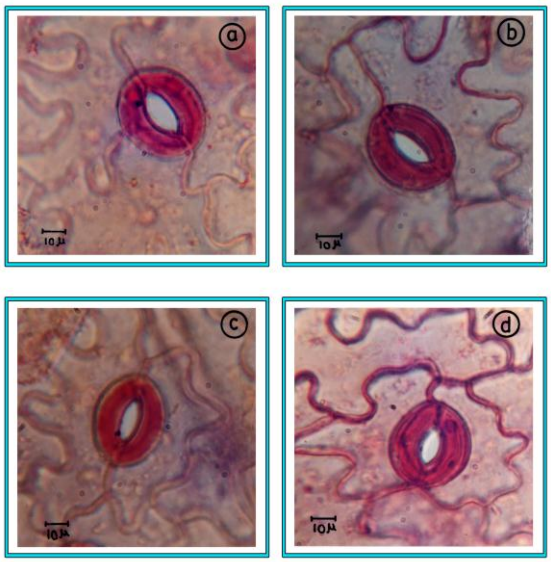

Plate-1: The effect of aqueous SO_2 on stomatal behaviour of isolated abaxial epidermal strips of pigeonpea (epidermal strips were stained with neutral red after 4 h of incubation), a - 0 ppm (10x100); b - 30 ppm (10x100); c - 100 ppm (10x100); d - 250 ppm (10x100).

In amaranth the stomatal aperture size of 10 ppm aqueous SO_2 incubated epidermal strips showed a slight increase in the first hour thereafter followed by a decline. All the other SO_2 concentrations decreased the stomatal aperture size with increasing duration of exposure period. The 250 ppm aqueous SO_2 concentration affected the stomatal aperture size to a maximum extent among the treatments. The stomatal aperture of the

corresponding controls increased continuously with increasing duration of exposure (Figure-1b; Plate-2).

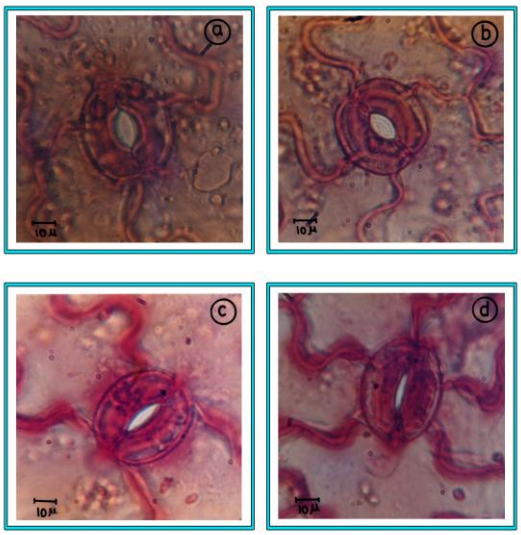

Plate-2: The effect of aqueous SO_2 on stomatal behaviour of isolated abaxial epidermal strips of amaranth (epidermal strips were stained with neutral red after 4 h of incubation), a - 0 ppm (10x100); b - 30 ppm (10x100); c - 100 ppm (10x100); d - 250 ppm (10x100).

The stomatal behaviour of pigeonpea and amaranth under aqueous SO_2 exposure were studied in isolated epidermal strips of their abaxial leaf surfaces. The time course of stomatal opening in isolated epidermal strips incubated in different concentrations of aqueous SO_2 showed, that lower aqueous SO_2 concentrations initially increased the stomatal opening, followed by a closure with increasing duration of SO_2 exposure (Plate-1, 2). However the SO_2 concentrations that were responsible for the stomatal opening differs in pigeonpea and amaranth. The stomatal opening was observed upto 30 ppm SO_2 in pigeonpea and at 10 ppm SO_2 in amaranth (Figure-1 a, b). The effect of SO_2 on the opening and closing responses of stomata were studied in detail in *Vicia faba* (Black and Unsworth, 1979b, 1980; Taylor *et al*., 1981). It was observed that low concentration of SO_2 induced stomatal opening probably due to the enhanced osmotic potentials and turgor of the guard cells (Biggs and Davis, 1980; Suwannapinut and Kozlowski, 1980). However, when epidermal strips were exposed to high SO_2 concentrations particularly for long periods, a conspicuous stomatal closure was observed (Bonte *et al*., 1977). This adverse stomatal closure may be due to the loss of turgor of the guard cells and the damage to the guard and subsidiary cells (Kimmer and Kozlowski, 1981; Agrawal and Deepak, 2003; Li *et al*., 2007).

Potassium distribution in guard cells

Histochemical technique was followed to study the distribution of potassium in the guard cells. The blackened areas formed as a consequence of cobalt sulphide, marked the positions of the potassium localisation and they were scored as described in the materials and methods section.

In pigeonpea, the score of blackened areas (precipitates) were more in 0 and 10 ppm followed by a continuous decrease with the increasing aqueous SO_2 concentration after 2 h of incubation. However, in amaranth the score of the blackened areas continuously decreased in the guard cells with increasing aqueous SO_2 concentration after 2 h incubation (Table-1).

Table-1: The effect of aqueous SO_2 on the degree of black cobaltous sulphide precipitate formed in the guard cells after 2 h incubation of isolated epidermal strips in aqueous SO_2 (The plus number indicate the intensity of potassium accumulation).

SO_2 conc. (ppm)	Plus numbers	
	Pigeonpea	Amaranthus
0	+ + + + + + +	+ + + + + + + +
10	+ + + + + + + +	+ + + + + + +
20	+ + + + + +	+ + + + + +
30	+ + + + +	+ + + + +
40	+ + + +	+ + + +
50	+ + +	+ + +
100	+ +	+ +
250	+	+

The histochemical localization of potassium in the isolated epidermal strips of pigeonpea after 2 h of incubation in aqueous SO_2 indicated that the guard cells of control and 10 ppm SO_2 treated epidermal strips exhibited higher quantities of potassium accumulation than the guard cells exposed to higher concentrations of SO_2 (Table-1). On the other hand, in amaranth the guard cells of control epidermal strips showed higher potassium levels than SO_2 treated ones at 2 h of incubation. These reasons suggest that SO_2 in probably involved in potassium fluxes and effects stomatal mechanism. Responses of plants vary between different species and their cultivars. Responses of plants to air pollutants also depend on type of pollutants, concentrations duration and its magnitude (Richa Rai *et al.*, 2011). Further the data indicated that amaranth appears to be more sensitive to SO_2 than pigeonpea.

CONCLUSION

The time course of stomatal opening in isolated epidermal strips of pigeonpea and amaranth showed that lower aqueous SO_2 concentrations initially stimulated the opening of stomata for a short period followed by a continuous decline with increasing period of exposure. Higher concentrations of SO_2 led to stomatal closure even from the beginning. However, the stomatal opening in pigeonpea and amaranth differed with respect to different concentrations. The histochemical distribution of potassium in the guard cells of isolated epidermal strips showed a close correlation between stomatal behaviour and potassium distribution in response to increasing concentrations of SO_2. The effect of aqueous SO_2 on stomatal behaviour revealed that

pigeonpea is relatively more tolerant and better equipped in combating SO_2 effect than amaranth.

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