STOMATAL RESPONSES OF *CAJANUS CAJAN* (C_3) AND *AMARANTHUS PANICULATUS* (C_4) PLANTS EXPOSED TO VARYING DOSES OF SULPHUR DIOXIDE BY SCANNING ELECTRON MICROSCOPY

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Abstract— Contamination of air due to release of phytotoxic pollutants such as sulphur dioxide (SO_2) may influence the composition of environment. Diffusion of gases into a leaf is hindered by several barriers. Stomata can regulate entry of a gas into the interior of a leaf. SO_2 enters in plants through open stomata, the gas reacts with moisture and is converted into acid. The acidic ions cause toxicity to the plant tissue. The effect of varying concentrations of aqueous SO_2 (0, 10, 20, 30, 40, 50, 100 and 250 ppm) on pigeonpea (*Cajanus cajan* (L.) Millsp. cv. PDM1), a C_3 plant and amaranth (*Amaranthus paniculatus* L. a local cultivar), a C_4 plant were selected for the present investigation of their leaf number, leaf area ratio and stomatal responses by scanning electron microscopy (SEM). The leaf number was reduced in both pigeonpea and amaranth in response to SO_2 exposure. The leaf area ratio of SO_2 treated plants was observed more in pigeonpea than in amaranth. The damage of stomatal complex at the necrotic regions and collapsed stomata were also observed at 250 ppm was evident from SEM studies in response to SO_2. Comparatively the reduction was more in amaranth than in pigeonpea.

Index Terms—Air pollutant, *Amaranth,* Aqueous SO_2, *Cajanus cajan,* leaf area ratio, leaf number, stomatal complex.

1 INTRODUCTION

Rapid industrialization and vehicular traffic especially in the urban areas of India is a great threat to air quality. The identification and categorization of plants into sensitive and tolerant groups is important because the former can serve as indicators and the latter as sinks for the air pollutants in urban and industrial habitats. The degradation of air quality is a major environmental problem that affects many urban and industrial sites and the surrounding regions worldwide. Plant distribution, all over the globe, is dependent on the mode of interaction of plants with their surrounding environment, which in turn depends on the type of environment and the degree of sensitivity or resistance of plants to the environmental stress (Dwivedi and Tripathi, 2007; Tripathi and Gautam, 2007). Sulphur, an essential element for all living plants, is taken up by plants in the form of sulphate from the soil through roots. Additional sulphur, if required, can be obtained by plants from the atmosphere, mostly in the form of SO_2, through leaf stomata (Khan *et al*., 2006).

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High SO_2 concentrations are phytotoxic and disturb stomatal behavior, photosynthesis and transpiration (Agrawal, 2003; Wali *et al*., 2004). In SO_2-exposed plants, sulphur accumulation occurs mainly in the aerial parts through open stomata on leaves (Iqbal *et al*., 2005; Mandal, 2006). In the mesophyll, SO_2 readily dissolves in aqueous phases thereby forming sulphurous acid with dissociation products as sulphite, bisulphite and protons (Rennenberg and Polle, 1994; Rennenberg and Herschbach, 1996). The sulphite and bisulphite anions are phytotoxic.

Leaves are the main photosynthetic organs of plants. Any alteration or disturbance in leaf morphology and metabolism would affect the growth of plant. Sulphur dioxide at phytotoxic concentrations affects the biochemical activities of the leaf, long before any visible injury appear on the leaves. Sulphur dioxide reduces leaf number, length and size accelerates leaf senescence as in *Phleum pratense* (Jones and Mansfield, 1982), *Vicia faba* (Kropff *et al*., 1989; Kropff, 1990), rye grass (Bell and Clough, 1973), *Nicotiana tobacum* (Mejstrik, 1980) winter barley (Pande and

Mansfield, 1985b), *Trifolium* (Murray, 1985a) and *Eucalyptus* (Murray and Wilson, 1988a, b). The concentration and duration of sulphur dioxide exposure and associated environmental factors such as light and humidity profoundly influence the behaviour of stomata. Sulphur dioxide may stimulate the opening of stomata at lower gaseous concentrations such as 25 ppb (Majernik and Mansfield, 1970; Unsworth *et al.*, 1972; Singh *et al*., 1985). Stimulatory effect of sulphur dioxide on stomatal opening has been reported for pine, *Phaseolus*, radish, sunflower, tobacco, field bean (Farrar *et al*., 1977; Aschenden, 1978; Rist and Davis, 1979; Black and Unsworth, 1980) and birch (Biggs and Davies, 1980). This stimulatory effect increased stomatal conductance and transpiration to a considerable extent. Closure of stomata subjected to higher SO_2 concentrations were noted by several workers Menser and Heggested (1966), Sij and Swanson (1974), Bonte *et al*. (1977), Qifu and Murray (1993). Higher concentrations reduce the viability of guard cells and damage the integrity of the guard cell chloroplasts (Black and Black, 1979a). Higher concentrations of gaseous SO_2 such as 0.25 ppm lead to conspicuous increase in stomatal opening due to extensive destruction of epidermal cells adjacent to the stomata (Black and Black 1979a; Black and Unsworth,1979b). Similar observations were made by Unsworth *et al*. (1972) in *Zea mays* leaves.

From the studies of how plants respond to severe stresses, we learn more about metabolism, its flexibility, its limits, and its diversity (Bohnert *et al*., 1995). Though certain direct effects of SO_2 on plant growth and metabolism are available, its effects on different plant species and its impacts on plant cellular structures are still needs further investigation. Therefore, the present study is intended to understand the effect of SO_2 on leaf number, leaf area ratio and stomatal changes in pigeonpea and amaranth.

2 MATERIALS AND METHODS

2.1 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).

2.2 Plant material

Pigeonpea (*Cajanus cajan* (L.) Millsp. cv. PDM), a C_3 plant is an important pulse crop of India. The seeds of pigeonpea are rich in protein content and are commonly used as source of vegetable protein in daily dietary intake of Indians. Being a legume, it fixes nitrogen and enriches soil fertility. And also it is profitable crop in India. Amaranth (*Amaranthus paniculatus* L.), a local cultivar), a C_4 plant is popular green leafy vegetable consumed all over India. Seeds of pigeonpea and amaranth were washed with distilled water and surface sterilized with 0.01 M mercuric chloride and were raised in earthen pots filled with soil containing farm yard manure and soil in the ratio of 1:3. The plants were watered on alternate days. The plants were grown under a natural photoperiod of approximately 12 h and average day temperatures of 31 ± 2 ^oC and 21 ± 1 ^oC at night at Andhra university experimental farm. The aqueous SO_2 at concentrations of 0, 10, 20, 30, 40, 50, 100 and 250 ppm was supplied as foliar spray at 8.00 a.m on every third day starting from five days after germination and continued up to one month. The zero SO_2 concentration treatment was called as control. The data were collected at weekly intervals starting from the day of foliar spray. The plants were separated into leaves, stems and roots prior to each analysis. The data were expressed on whole plant, per part and on unit fresh weight and/or dry weight basis. The contents expressed for the whole plants were obtained by summation of the individual parts.

2.3 Leaf number: The number of leaves was counted for 1, 2, 3 and 4 week old plants after SO_2 treatments.

2.4 Leaf area and leaf area ratio (LAR): Immediately after separation of leaves from the stem, the samples of leaves from each plant were placed on a mm graph paper. The outline of the leaves were marked with a pencil and the leaf area of the leaves was calculated. The LAR was obtained as the leaf area of the

plant divided by dry weight of the plant and it was expressed as cm^2 mg^{-1} (Murray and Wilson, 1988a).

2.5 SEM studies of leaf surface: The selected third leaf from the top of the 3-week old treated plants fixed in 2.5 % glutaraldehyde in 0.025 M phosphate buffer, dehydrated with alcohol series and then subjected to critical point drying in solid carbon dioxide. Ten mm of the dried specimens were coated with gold palladium and examined in scanning electron microscope (JEOL-JSM-T330A).

3 RESULTS AND DISCUSSION

The number of leaves increased with increasing age in both pigeonpea and amaranth plants. The leaf number of control plants was always higher than the treatments. The decrease in leaf number of treated plants became more conspicuous with increasing aqueous SO_2 concentration. The reduction in leaf number was maximum at 250 ppm SO_2 treated plants in both the plant species. In between pigeonpea and amaranth, the reduction of leaf number in response to SO_2 was more pronounced in amaranth (Figure-1a,b).

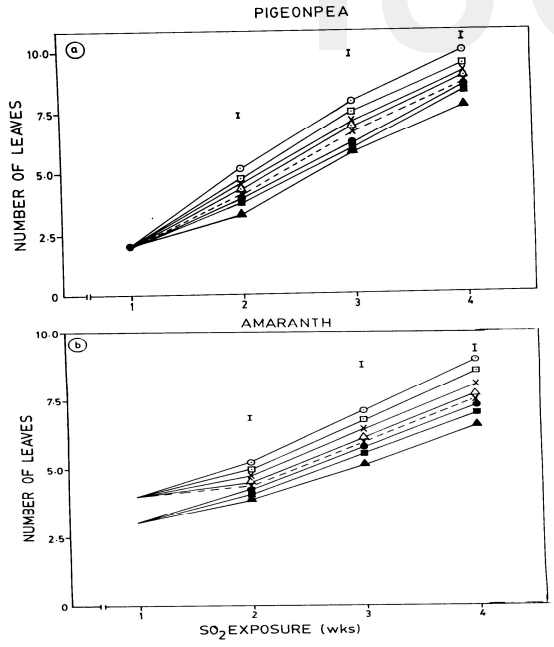

Figure-1: The effect of foliar application of aqueous SO_2 on number of leaves of pigeonpea (a) and amaranth (b) (vertical lines represent S.E.)
○- 0 ppm; □-10 ppm; ×-20 ppm; Δ-30 ppm; ×--- 40 ppm; ●-50 ppm; ■-100 ppm; ▲-250 ppm.

Sulphur dioxide exposure of plants reduced the number of leaves and accelerated leaf senescence in rye grass and in *Phleum pratense* (Mansfield and Jones, 1985). Measurements of leaf number was made at weekly intervals both in pigeonpea and amaranth. The first and second leaves expanded normally in control as well as in lower concentrations of SO_2 treated plants both in pigeonpea and amaranth. However at higher SO_2 concentrations, initiation and development of leaves were delayed. The leaf number in both pigeonpea and amaranth decreased under SO_2 exposure (Figure-1a,b). The reduction of leaf number was more in amaranth than in pigeonpea in response to aqueous SO_2 exposure. It was proposed that the delay in translocation of photosynthates to the meristematic sinks might have delayed the leaf initiation in SO_2 treated plants (Murray, 1985a; Tanvir Ali *et al*., 2008).

Leaf area ratio of pigeonpea and amaranth differed with age. The leaf area ratios of the pigeonpea showed a gradual decline from the first week to the third week and remained almost constant up to the fourth week. The SO_2 treated plants always recorded higher values than the control. The maximum leaf area ratio was observed in 250 ppm SO_2 treated plants and the minimum values in the control plants (Figure-2a,b).

The leaf area ratio (LAR) is the proportion of assimilatory area to the plants dry weight. It is considered to be one of the parameters that indicates plants adjustment to SO_2 exposure (Mansfield and Jones, 1985; Whitmore *et al*., 1985). The response of LAR to SO_2 exposure differed between pigeonpea and amaranth. The SO_2 treated plants of pigeonpea always exhibited higher LAR values over their respective controls (Figure-2a) similar to observations made on *Phleum pratense* (Whitmore and Mansfield, 1983; Mansfield and Jones, 1985). On the other hand, amaranth exhibited lower LAR values in SO_2 treated plants than their respective controls (Figure-2b) similar to *Eucalyptus calophylla* and *E. gamocephala* (Murray and Wilson, 1988 a). The changes in LAR could be considered as a part of the compensating mechanism to cope up with reduced photosynthetic efficiency under SO_2 exposure (Whitmore and Mansfield, 1983).

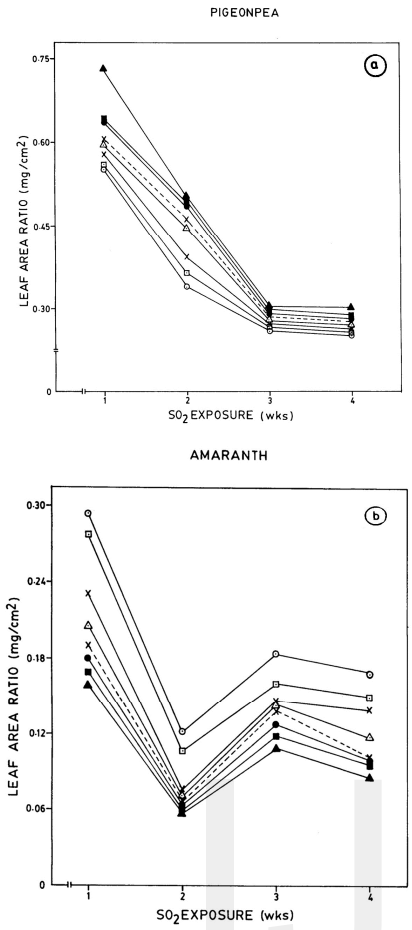

Figure-2: The effect of foliar application of aqueous SO_2 on the leaf area ratio of pigeonpea (a) and amaranth (b)
○- 0 ppm; □-10 ppm; ×-20 ppm; Δ-30 ppm; ×--- 40 ppm; ●-50 ppm; ■-100 ppm; ▲-250 ppm.

In amaranth the leaf area ratio of control and aqueous SO_2 treated plants recorded greater values on the first week of the plant growth touching a minimum with respect to each plant treatment and rising the values to a certain extent on the third week once, again followed by a decline on the fourth week. The leaf area ratio of all the SO_2 treated plants always remained lower than the control. The maximum values were always recorded in the control and the minimum values in 250 ppm aqueous SO_2 treated plants (Figure-2b).

Increasing aqueous SO_2 concentration application on both pigeonpea and amaranth affected the structure and organization of stomata.

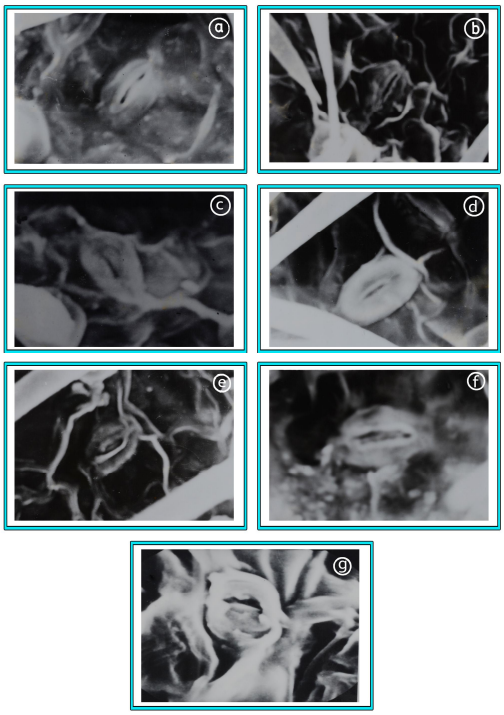

Plate-1: Scanning electron micrographs of pigeonpea, showing stomata of adaxial leaf surface of 3-week old plant, in response to the foliar application of aqueous SO_2.
a - 0 ppm (x1000); b - 30 ppm (x1000); c - 250 ppm (x1000); d - 0 ppm (x1000); e - 30 ppm (x1000); f - 250 ppm (x1000); g - 250 ppm (x1000).

The stomata of both pigeonpea and amaranth collapsed and lost their capacity of stomatal movement in response to SO_2 (Plate-1a,b,c,d,e,f,g; Plate-2a,b,c,d,e). Scanning electron micrographs (SEM) of the adaxial and abaxial leaf surfaces (third leaf) of pigeonpea and amaranth were shown in the Plates-1, 2. The SEM studies indicated that SO_2 induced wide opening of stomata in the leaves of SO_2 treated plants of both pigeonpea and amaranth. Collapse of stomatal complex at necrotic regions was also observed (Plate-1g, 2e) in both the plant species. It is suggested that SO_2 adversely effects the membrane permeability of guard and subsidiary cells resulting in the loss of their turgidity and damage to the stomatal complex which ultimately affects the stomatal mechanism (Majernik and Mansfield, 1972; Unsworth *et al.,* 1972; Black and Black, 1979a; Singh *et al.,* 1985; Neighbour *et al.,* 1988; Tanvir Ali *et al*., 2008). This phenomenon may impair gaseous exchange and transpiration in SO_2 treated plants.

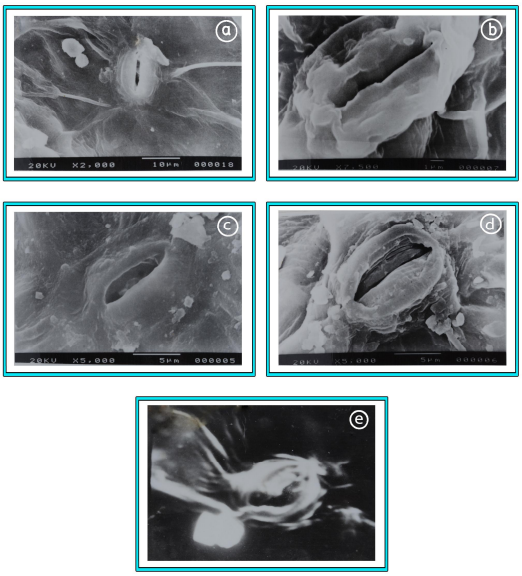

Plate-2: Scanning electron micrographs of amaranth, showing stomata of 3-week old plant, in response to the foliar application of aqueous SO_2.
a - 0 ppm Upper epidermis (x2000); b - 250 ppm Upper epidermis (x7500); c - 0 ppm Lower epidermis (x5000); d - 250 ppm Lower epidermis (x5000); e - 250 ppm (x1000).

4 CONCLUSION

The number of leaves were reduced in both the plant species studied in response to SO_2. The reduction in leaf number also dependant on SO_2 concentration and age, but it varies between pigeonpea and amaranth. The leaf area ratio differed between pigeonpea and amaranth in response to SO_2. The SO_2 treated pigeonpea plants exhibited higher leaf area ratio than their respective controls. In contrast to this SO_2 treated amaranth plants showed lower leaf area ratio than their respective controls throughout the study period. The changes in leaf area ratio are considered as a compensatory mechanism in response to reduced photosynthetic activity due to SO_2 injury. The SEM studies indicated the damage of stomatal complex specially at the necrotic regions.

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