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Ultrastructural Changes in *Malva sylvestris* Leaves in Response to Aqueous Sulfur Dioxide

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Summary

Leaf discs of *Malva sylvestris* were treated with different concentrations of aqueous sulfur dioxide (10-1000ppm) for four hours under illumination (500W tungsten bulb). Scanning electron microscopy was used to determine the extent of damage to the ultra structure of *Malva sylvestris* under sulfur dioxide exposure. Slight opening of stomata was observed at 100 ppm exposure and at 1000 ppm concentration of aqueous sulfur dioxide well pronounced opening of stomata was found, mesophyll cell collapse associated with cellular disorganization and plasmolysis was also observed.

Key Words: Aqueous sulfur dioxide, *Malva sylvestris*, Ultrastructure, Scanning electron microscopy.

Introduction

The effects of sulfur dioxide on vegetation have been well reviewed in terms of foliar injury (1,2,3) and physiological and biochemical alterations (4,5,6,7,8,9,10,11). However, the effects of sulfur dioxide on the subcellular structural organization are less known. Ultra structural evidence for sulfur dioxide induced effects was first provided by Wellburn et al. (12) and Pechak et al. (13) who reported reversible swelling of the thylakoid membranes of chloroplast in leaves exposed to low concentrations of sulfur dioxide. Sulfur dioxide has also been observed to influence the ultrastructure of conifer needles, especially the chloroplasts of mesophyll tissue adjacent to stomata (14).

Materials and Methods

Generation of aqueous sulfur dioxide

Sulfur dioxide was generated by reducing hot concentrated sulfuric acid with copper turnings and estimated according to West and Gaeke (15).

Exposure of leaf discs to aqueous sulfur dioxide

Malva Sylvestris was purchased from local market and. discs of 1 cm diameter each were cut from healthy leaves using a stainless steel cork borer. Leaf discs were treated with 10, 100 and 1000 ppm of aqueous sulfur dioxide for four hours in petri dishes (15 x 20 mm) under illumination which was provided by a 500 W electric bulb. Treatment conditions were kept similar for each section.

Scanning Electron Microscopy

Tissue fixation

Control and treated leaf discs of *Malva sylvestris* were fixed for eight hours at 4^{o}C in 2% glutaral dehyde prepared in 0.05 M sodium cacodylate buffer. After fixing, the samples were kept in sodium cacodylate buffer (washing buffer) overnight at 4^{o}C and then post fixed for 2-4 hours in 1% OsO_4 prepared in 0.05M sodium cacodylate buffer.

Dehydration

The leaf discs were washed briefly with distilled water and dehydrated in an increasing series of ethanol (50-100%), 10 minutes at each step, followed by two additional periods of absolute ethanol (10 min. each). The leaf discs were further dehydrated by critical point drying at 31^{o}C for 5–10 minutes.

Mounting specimen for SEM

Dried tissue was mounted on a specimen holder for the SEM and dried overnight in a vacuum desiccator. In the final stage before viewing, the samples were sputter coated with gold and examined in the S-3000H scanning electron microscope.

Results and Discussion

Under the electron microscope *Malva sylvestris* leaf in absence to any exposure of sulfur dioxide (control) showed no damage to the cell structure (Fig. 1). Intact stoma and cells were observed (Fig. 2). Leaf discs exposed to 10 ppm showed no stomatal response (Fig.3) while at 100 ppm of aqueous sulfur dioxide treatment stomatal opening was observed (Fig. 4,5). Black and Black (16) used light microscopy to examine epidermal strips taken from bean plants exposed either to scrubbed or to polluted air. The enhanced opening response induced by low concentration of sulfur dioxide was associated with extensive destruction of adjacent epidermal cells whereas the guard cell survival was not reduced significantly. Stomatal effects induced by sulfur dioxide are varied in magnitude and direction. Depending upon the species and the environmental conditions, exposure to sulfur dioxide may result in stomatal closure, stomatal opening or no reaction of stomata at all (17). The leaf discs exposed to 1000ppm of sulfur dioxide showed

well pronounced opening of stomata. Cellular disorganization, plasmolysis and reduced guard cell visibility was also observed (Fig. 6, 7). Disruption of inner structure was also clear. Similar results were obtained when *Spinaceae oleraceae* was exposed to varying concentrations of aqueous sulfur dioxide (18).

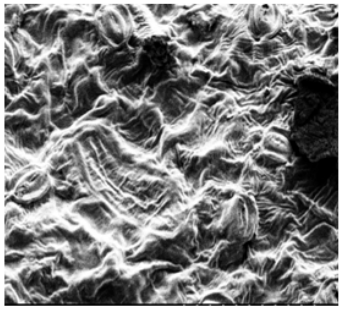

Fig.1: Surface morphology of *Malva sylvestris* leaf

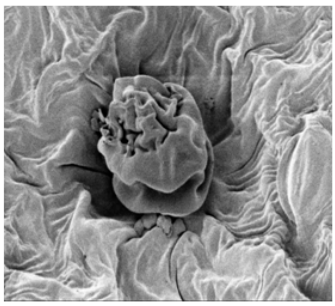

Fig.2: Intact stomata in control *Malva sylvestris* leaf

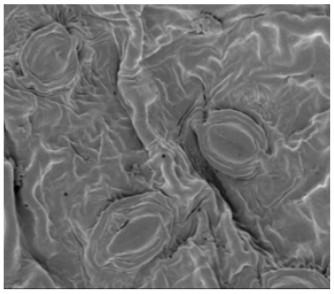

Fig.3: Intact stomata of *Malva sylvestris* leaf exposed to 10 ppm of aqueous sulfur dioxide

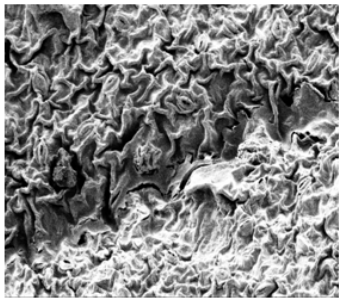

Fig.4: Surface morphology of *Malva sylvestris* leaf exposed to 100 ppm of aqueous sulfur dioxide

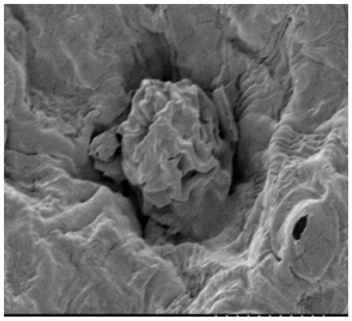

Fig.5: Opened stomata in *Malva sylvestris* leaf exposed to 100 ppm of aqueous sulfur dioxide

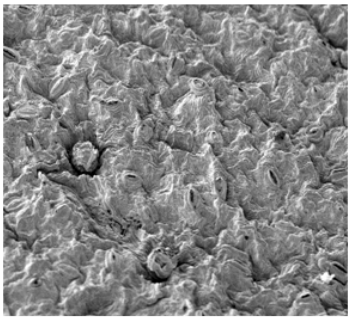

Fig.6: Surface morphology of *Malva sylvestris* leaf exposed to 1000 ppm of aqueous sulfur dioxide

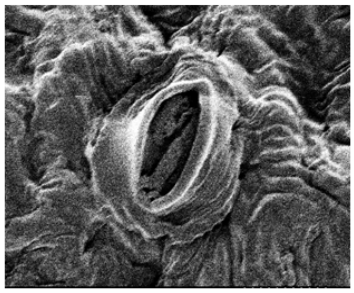

Fig.7: Openend stomata of *Malva sylvestris* leaf exposed to 1000 ppm of aqueous sulfur dioxide

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