Leaf Micromorphology of Zinc Deficient Field Bean

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Light and Scanning electron microscopic examination of leaf morphology and topography of zinc sufficient field bean plants grown with 65 µg Znl^{-1} and zinc deficient plants grown with 1.3 µg Znl^{-1} showed zinc effect on degree of wax depositon, shape and size of stomatal complex and the extent of stomatal opening. The leaves of the zinc deficient plants had ringed flaccid guard and accessory cells,sunken stomata with distorted stomatal lips, and excessive deposition of epicuticular wax. The number and size of the stomata was significantly reduced by zinc deficiency. Zinc deficiency also caused partial closure of the stomata. These changes were reflected in increase in diffusive resistance associated with decrease in transpiration rate of Zn deficient plants.

Key-words - Zinc deficiency, leaf morphology, stomata.

INTRODUCTION

ZINC deficiency affects leaf morphology and induces water stress in plants (Sharma *et al.* 1982; Sharma & Sharma 1987). In cabbage, zinc deficiency leads to thickening of lamina, heavy deposition of epicuticular wax and closure of stomata that reduces water loss (Sharma *et al.* 1982). The objective of this paper was to investigate zinc deficiency induced changes in surface morphology and stomatal condition as revealed under scanning electron microscope (SEM) and associated change in leaf diffusive resistance and transpiration of field bean, widely cultivated for edible pods in zinc deficient soils of India (Katyal & Sharma 1991).

MATERIAL AND METHODS

Field bean (*Vicia faba* L.) was grown in glass house in refined sand (Agarwala and Sharma 1976) with sufficient (65 µg Znl^{-1}) and deficient (1.3 µg Znl^{-1}) zinc. Between 50 to 65 d, when zinc deficient plants had just developed visible symptoms of zinc deficiency, light and scanning electron microscope (SEM) studies were made of surface morphology and stomata of comparable (top 3rd to 5 th) leaves of zinc sufficient and zinc deficient plants. Stomatal frequency and size were measured in micro relief impression (Sharma et al. 1982) of the adaxial and abaxial surface of the amphistomatous leaves.

For scanning electron microscopy (SEM) plant material was cut into 1 mm pieces and fixed for 18 h with 3% glutaraldehyde in 0.025 M phosphate buffer (pH 6.8). After three washes with phosphate buffer, the sample were post fixed for 2 h in 1% (w/v) osmium tetroxide in the same buffer. The sample were dehydrated through ethanol-isoamly series, dried with liquid CO_2 in critical point dryer (CPD),mounted on stubs and coated with gold in a sputter coater. All specimens were observed in a JEOL(JSM-35C) scanning electron microscope (SEM) and representative areas of leaf surface were photographed at accelerating voltage of 20 KV.

Stomatal diffusive resistance and rate of transpiration were measured in intact leaves using a LI-COR model LI-1600 (LI- COR,Lincoln, Neb.) steady state porometer under glass house conditions between 9 AM and 10 AM, with temperature ranging 25° to 30 °C and humidity 60 to 70% under high intensity PAR 700 to 900 $\mu E\, m^{-2} s^{-1}$.

RESULTS AND DISCUSSION

Compared to zinc sufficient plants, leaves of zinc deficient plants were small, thick, leathery and brittle. The lamina appeared dul!, lacking lustre. Microrelief impressions of the epidermal peelings showed reduction in stomatal frequency and size, in leaves of zinc deficient plants. The effect was induced earlier and was more marked on the abaxial than on the adaxial surface

Table 1. Effect of zinc deficiency on stomatal frequency, size and stomatal aperture, transpiration and diffusive resistance in leaves of field beans at 50 and 65 days of growth.

Zinc in nutrient solution ($\mu g\ Zn\ l^{-1}$)	Stomata				Stomatal aperture (μm)		Transpiration rate (μg) $cm^{-2}\ s^{-1}$)	Diffusive resistance ($s\ cm^{-1}$)
	Frequency stomata/mm		Size LxB (μm)					
	Ad	Ab	Ad	Ab	Ad	Ab		
			50 d					
65	42	56	43.2x27.4	45.1x36.1	4.76	3.54	4.08	6.97
1.3	52	47	42.1x25.4	43.2x26.7	2.36	2.15	21.6	8.62
			65 d					
65	45	58	45.2x26.6	46.8x37.4	4.91	3.52	4.05	6.63
1.3	56	42	37.2x26.1	36.1x28.8	0.91	1.50	1.54	12.14

Ad- Adaxial, Ab - Abaxial

(Table 1). Leaves of zinc sufficient plants had higher frequency of stomata on abaxial than on adaxial surface. Zinc deficiency caused increase in stomatal frequency on the adaxial surface but decreased the stomatal frequency on abaxial surface. As compared to zinc sufficient plants, stomata of zinc deficient plants were smaller in size (Table 1). Compared to zinc sufficient plants, a large number of stomata remained partially open to closed in zinc deficient plants and this was reflected in marked decrease in transpiration rate and increase in diffusive resistance which became more pronounced with increase in age of zinc deficient plants (Table 1).

The effect of zinc supply on the stomatal opening in the two leaf surface varied with age. In adaxial surface of zinc deficient plants few stomata were closed at 50d (Figs. 1-8) and the extent of opening was less than in zinc sufficient the plants (Pl. 1, Fig. A). At 65 d almost all (90%) of stomata failed to open (Pl.1, Fig C). On the abaxial leaf surface of zinc deficient plants, very few (30%) stomata were open and the extent of opening of stomata (pore width) was less than in zinc sufficient plants (Table 1). SEM observations revealed thick deposition of epicuticular wax on the epidermal cells of zinc deficient plants giving the cells a well defined outline. The deposition of wax was more on the adaxail surface and became more pronounced at later stages of deficiency (Pl. 1, Fig. C). Because of the deposition of wax, the stomata of zinc deficient leaves appeared sunken (Pl.1, Figs. B, C, E, F) as compared to stomata of zinc sufficient plants (Pl. 1, Figs A, D).

In zinc deficient plants the partially open to, close stomatal lips were also flaccid and distorted (Plate 1, Figs E, F, H, I) as compared to the fully open and turgid stomata of zinc sufficient plants (Plate 1, Figs D, G). The guard cells of zinc deficient stomata showed loss of turgidity with distinct folds or wrinkles, not only at the margins but also in the adjoining subsidiary cells (Plate 1, Figs E, H). As deficiency prolonged, these became more pronounced, leading to characteristic ringed and flaccid guard cells (Plate 1, Figs F, I). Earlier, Humble and Raschke (1971) had demonstrated that guard cell volume and osmotic pressure decreased in the closed stomata of faba beans due to efflux of K^+ from the guard cell. Our study provides evidence to suggest that zinc deficiency induced change in leaf morphololgy (leaf thickness and epicuticular wax depositions) and stomatal structure (flaccid ringed guard cells and closed stoma), cause marked decrease in transpiration and increase in diffusive resistance. This is in consonance with observations of our earlier study (Sharma *et al.* 1987; Pandey & Sharma 1989).

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Plate 1 →

Figs A-I. Scanning electron micrographs of the leaf surface of zinc deficient (1.3 $\mu g\ Zn\ l^{-1}$) and zinc sufficient (65 $\mu g\ Zn\ l^{-1}$) field bean plants. Adaxial surface of leaves of zinc sufficient (A) and zinc deficient plants at initial (B) and acute stages of deficiency(C). Abaxial surface of zinc sufficient plant (D) and zinc difcient plants at initial (E) and acute stages (F). Enlarged view of stomata on abaxial surface surface on zinc sufficient (G) and zinc deficient plants (H,I).

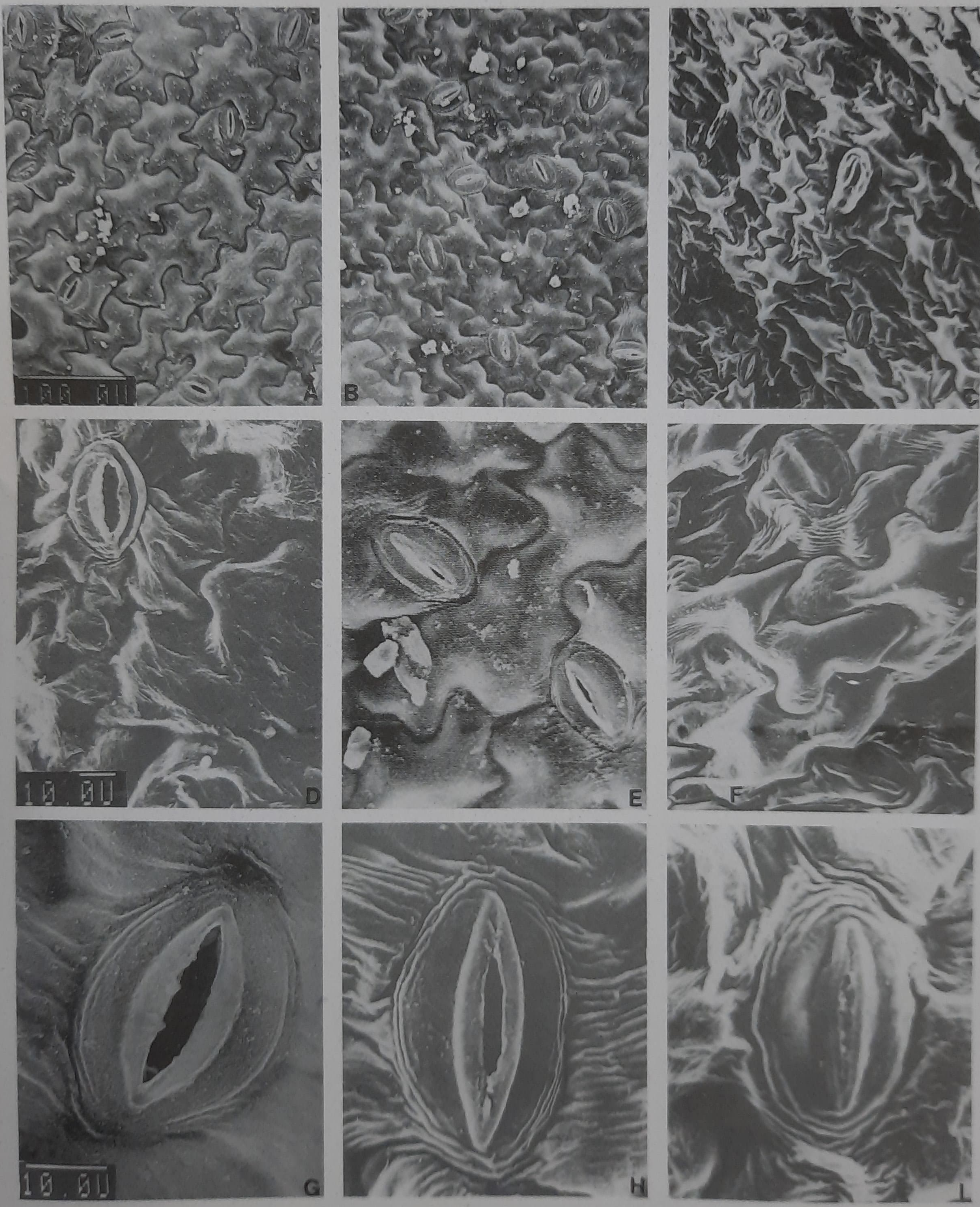

Plate 1

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