Ultrastructural changes in the pollen grains of green gram subjected to copper deficiency

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Copper deficiency stress caused structural changes in the pollen grains of green gram (*Vigna radiata* L. cv T-44) that led to pollen sterility. Supply of adequate Cu to the Cu-deficient plants led to partial reversal of the ultrastructural changes in the pollen grains and an improvement in the *in vitro* germination.

Key-words : Cu deficiency, pollen grains, green gram.

INTRODUCTION

COPPER deficiency is known to delay flowering and induce pollen sterility. Graham (1976) suggested that a breakdown of microsporogenesis at or near meiosis resulted in poor pollen fertility. Dell (1981) found that poor lignification of the endothecial layers of anthers of Cu-deficient plants prevents normal anther dehiscence. Jewell *et al.* (1988) observed that Cu deficiency stress results in abnormal development of tapetum leading to sterility of pollen grains. In this paper we describe the effect of Cu deficiency stress on the ultrastructure and viability of pollen grains.

MATERIALS AND METHOD

Green gram (*Vigna radiata* L. cv. T-44) was raised in refined sand with 0.063 mg Cu L^{-1} (Cu-adequate) and 0.0063 mg Cu L^{-1} (deficient) Cu supply. The composition of the nutrient solution and the method of sand, nutrient and water purification was as described earlier (Sharma *et al.*, 1987). Thirty days after sowing (30 DAS), when plants raised with 0.0063 mg Cu L^{-1} had developed visible symptoms of Cu deficiency stress, pots of plants receiving 0.0063 mg Cu L^{-1} were separated into two lots. While Cu-deficient supply was maintained to one lot, the other lot was supplied adequate (0.063 mg L^{-1}) Cu. The three sets of plants viz. (a) Cu-sufficient plants, (b) Cu-deficient plants and (c) Cu-deficient turned Cu-sufficient plants, were studied for ultrastructure and viability of pollen grains. The flower buds of (a), (b) and (c) were fixed in Formalin-Acetic Acid- Alcohol (FAA) at the time of anthesis. Pollen viability was determined by *in vitro* germination of pollen grains using hanging drop technique (Brewbaker & Kwack 1963). For light microscopic study of pollen structure, pollen grains were subjected to acetolysis (Erdtman 1952) and mounted in glycerine jelly. For SEM studies, the acetolysed samples of pollen grains were dehydrated through alcohol series and mounted on brass stubs. The stubs were coated with gold-palladium in a sputter coater and examined in Jeol-JSM-35C SEM at an accelerated voltage of 10 KV.

RESULTS

Copper deficient plants of green gram (*Vigna radiata* L. cv T-44) showed depression in growth and developed characteristic Cu-deficiency symptoms like bluish green lustre, cupping of young trifoliates 30 DAS. Compared to Cu-sufficient plants, in Cu-deficient plants flowering was delayed by 10 days. The flower size of Cu-deficient plants was also reduced. Copper deficiency caused decrease in the size and pore diameter of pollen grains, but increase in the thickness of exine (Table 1). The Cu-deficient plants that were turned Cu-sufficient, produced pollen grains that had a larger size and larger pore diameter than the pollen grains of Cu-deficient plants, but these were still less than in Cu-sufficient plants. The thickness of the exine was also reduced by supply of adequate Cu to the Cu-deficient plants but it still remained more than that in pollen grains of Cu-deficient plants (Table 1). Compared to Cu-sufficient plants, pollen grains of Cu-deficient plants showed poor *in vitro*

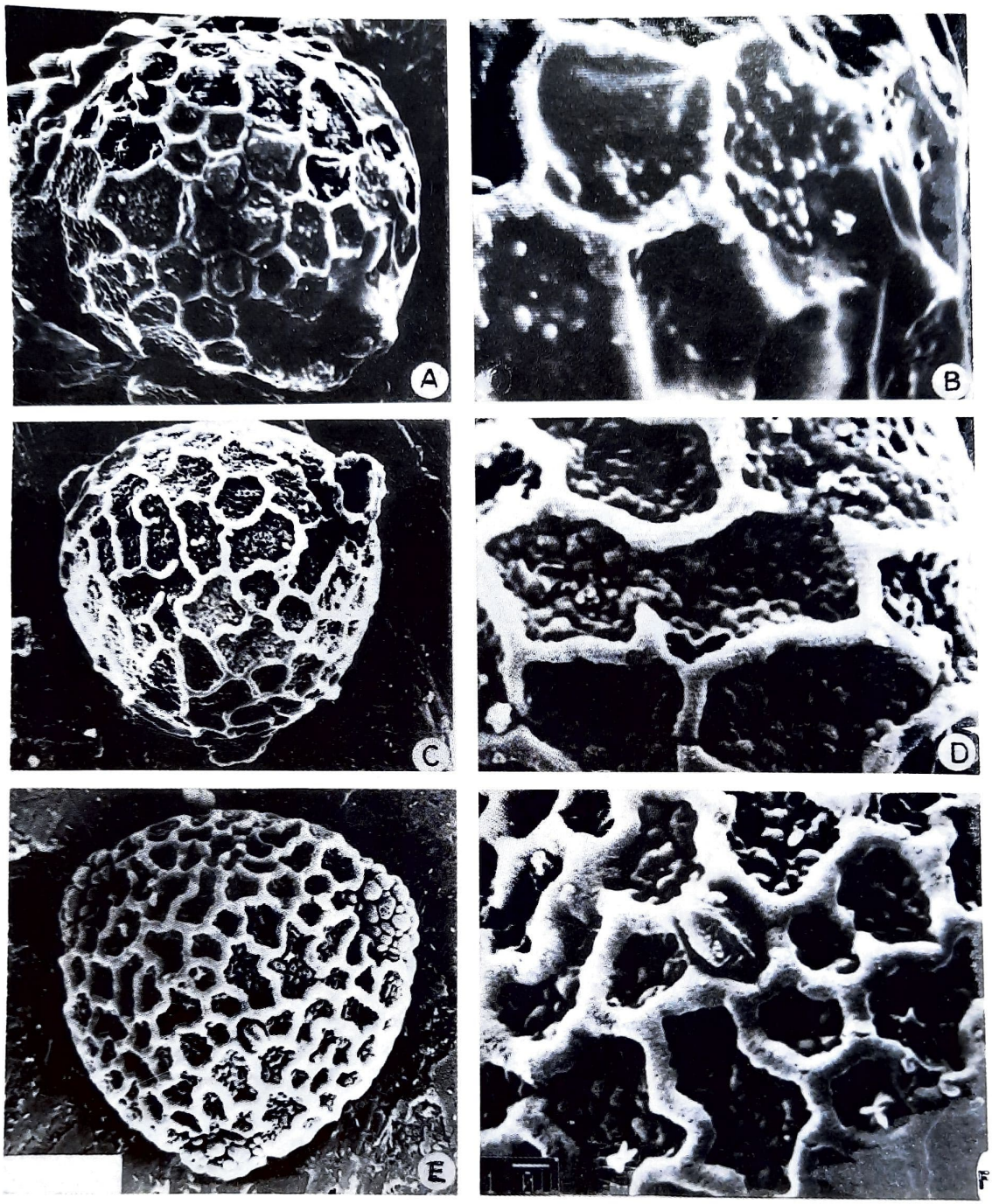

Plate 1

1. Sanning Electron Micrographs of pollen grains of Cu-sufficient (A,B), Cu-deficient (C,D) and Cu- deficient turned Cu-sufficient (E,F) plants of *Vigna radiata* L. cv. T-44.

germination. After these plants were turned Cu-sufficient the pollen viability showed a slight increase (Table 1).

Copper deficiency stress led to alterations in the pattern of ornamentation of the exine. Copper deficient pollen grains showed large cells with irregular shape forming an imperfect reticulum. In contrast to this, Cu-sufficient plants showed a perfect reticulum with penta- to hexagonal cells with thin and straight muri. The lumen of these pollen grains had few sexinous elements and the operculum was smooth with few opercular granules (Plate 1 A, B). The exine of the Cu-deficient pollen grains showed lumen with pilate projections of the baculae and muri that were thick with irregular and

Table 1 : Effect of Cu deficiency on the pollen size, exine thickness and *in vitro* germination of pollen grains of *Vigna radiata* L. cv T- 44.

Cu in nutrient solution (mg L^{-1})	Pollen diameter (µm)	Pore diameter (µm)	Exine thickness (µm)	*In vitro* germination of pollen (%)
0.063	39.06	5.44	3.41	65
0.063	36.40	5.16	3.56	35
0.0063 + 0.063	38.71	5.27	3.52	50

wavy walls (Plate 1 C, D). Supply of adequate Cu to Cu-deficient plants produced pollen grains that resembled the pollen grains of Cu-sufficient plants due to the compact reticulate pattern of the exine. Unlike the pollen grains of Cu-deficient plants, the pollen grains of Cu-deficient turned Cu-sufficient plants, showed a reticulum with compactly arranged cells of varying size. However, unlike the pollen grains of Cu-sufficient plants the muri was thick and wavy, enclosing pilate projections of the baculae (Plate 1 E, F).

DISCUSSION

Copper deficiency stress not only retarded vegetative growth of plants and produced visible symptoms of Cu deficiency but also affected the reproductive development of plants. Deficiency of Cu led to delay in the onset of reproductive phase, produced structural changes in the pollen grains and induced pollen infertility. These changes suggest a role of Cu in the development of the pollen grains. As observed in the present study, reduction in size of pollen grains and decrease in the *in vitro* germination has also been reported in case of Cu-deficiency in wheat by Graham (1975) and Agarwala *et al.*, (1980). Agarwala *et al.* (1980) suggested poor metabolism associated with increase in ribonuclease and decrease in oxido- reductases resulting in loss of respiratory O_2 for pollen tube growth as a cause of reduced viability of pollen grains in Cu-deficient wheat plants. In green gram, we found that Cu-deficient pollen grains are not only reduced in size but also undergo structural impairment such as increase in exine thickness and formation of thick muri and pilate baculae. Such structural abnormalities would contribute to loss of pollen viability. Jewell *et al.* (1988) had reported that in Cu-deficient plants, instead of supplying the pollen with nutrients, the tapetum becomes expansionary and hypertrophied. This expansive nature of the tapetum leads to disruption of exine formation and abnormal pollen development. The present study revealed that Cu-deficiency stress induces structural abnormalities in the exine that are associated with infertility of the pollen grains and points to a structural and functional role of Cu in pollen biology.

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