Zinc Deficiency Affects Pollen Structure and Viability in Green Gram (*Vigna radiata* L. *cv T-44*)

N Pandey, M Gupta & C P Sharma

Department of Botany, Lucknow University, Lucknow 226 007

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Green gram (*Vigna radiata* L. *cv T-44*) was grown with zinc sufficient (0.065 mg Zn L^{-1}) and zinc deficient (0.0065 mg Zn L^{-1}) nutrient solution in refined silica sand and examined for zinc effect on structure and viability of pollen grains. Compared to pollen grains of Zn sufficient plants, pollen grains of Zn deficient plants were smaller in size, had reduced pore size and increased thickness of exine. Study of ultrastructure of pollen revealed that while exine of Zn sufficient plants had reticulate ornamentation with uniform muri, the exine of Zn deficient pollen grains had irregular muri with heavy deposition of wax and sexinous particles in the lumen. The Zn deficiency stress induced changes in the pollen structure are suggested to reduce viability of the pollen grains.

Key-words–Zn deficiency, pollen grains, green gram.

INTRODUCTION

ENVIRONMENTAL factors which determine the growth of the plants also affect the pollen performance in terms of pollen producing capacity, pollen size and the viability of pollen grains. This has been reported to be so in case of high and low temperature (Saini & Aspinall 1982; van Herpen 1986), air pollutants (Wolter & Martens 1987) and mineral stress (Vasek 1987; Young & Stanton 1990; Lau & Stephenson 1993; Pandey *et al*. 1995). Deficiency of zinc, one of the most widespread micronutrient disorders in Indian soils (Takkar 1991), has been shown to induce pollen sterility in wheat (Sharma *et al*.1979) and maize (Sharma *et al*. 1987; 1990). Pandey *et al*. (1995) described the Zn deficiency effect on pollen-stigma interaction in faba bean (*Vicia faba* L). This paper deals with the zinc deficiency effect on structure and viability of pollen grains of green gram (*Vigna radiata* L.) and the effect of making up the deficiency on the changes induced by the deficiency.

MATERIAL AND METHOD

Green gram (*Vigna radiadta* L. *cv T-44*) was raised in refined silica sand with 0.065 mg Zn L^{-1} (Zn sufficient) and 0.0065 mg Zn L^{-1} (Zn deficient). The composition of the nutrient solution and the methods of sand, nutrient and water purification have been described earlier (Sharma *et al.* 1987). At d 35 when Zn deficient plants developed visible symptoms of Zn deficiency, pots supplied 0.0065 mg Zn L^{-1} were separated into two lots. While supply of 0.0065mg Zn L^{-1} was continued to one of the two lots, the second lot of the Zn deificient pots was supplied Zn suficient nutrient solution (0.065 mg Zn L^{-1}).

The pollen grains were examined for the effect of Zn deficiency and recovery therefrom. For this purpose, the flower buds of (a) Zn sufficient plants, (b) Zn deficient plants and (c) Zn deficient plants subsequently made Zn sufficient were fixed in Formalin-Acetic-Alcohol (FAA) immediately before anthesis. The pollen grains were prepared by the acetolysis method of Erdtman (1986). After acetolysis, pollen grains were mounted in glycerine jelly for light microscopy. For SEM studies, the acetolysed samples of pollen grains were dehydrated in 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. Viability of pollen grains was determined by *in vitro* germination of pollen using hanging drop technique in the growth medium (Brewbaker & Kwack 1963).

RESULTS

Green gram plants subjected to Zn deficiency developed visible effects of the deficiency 35 DAS. These plants showed reduction in growth, shortening of internodes and reduction in size of leaflets. Compared to Zn sufficient plants, flowering of Zn deficient plants was delayed by 7 to 10 days.

The pollen grains of Zn deficient plants were smaller in size than the pollen grains of Zn sufficient plants (Table 1). Polar view of pollen grains of Zn sufficient plants show these to be 3-zonoporate, spherical with equatorial and circular pores. Zinc deificiency led to decrease in pore diameter and increase in exine thickness. Supply of 0.065 mg Zn L-[1] to plants grown with 0.0065 mg Zn L-[1], led to partial recovery from Zn deficiency effects on pollen size and thickness of the exine (Table 1). Compared to Zn sufficient plants, pollen grains of Zn deficient plants showed poor germination suggesting induction of pollen sterility. Supplying adequate Zn (0.065 mg Zn L-[1]) to Zn deficient plants led to improvement in pollen viability (Table 1).

The pollen grains of Zn sufficient plants had tectum showing perfect reticulate ornamentation due to penta-to hexagonal cells with straight and uniform muri and lumen filled with few sexinous elements. They also showed poor deposition of wax (Figure 1A). The pore of the pollen grains of Zn sufficient plants had a smooth operculum and possessed few large granules (Figure 1B). The Zn deficient pollen grains underwent changes in reticulate ornamentation showing large incomplete cell (Figure 1C), formed as a result of dissolution of the walls forming the reticulum. While exine of Zn sufficient pollen grains showed straight and smooth muri, the exine of Zn deficient plants showed thick, sinuous and lobed muri with particulate depositions (Figure 1D).

As the Zn deficient plants gained sufficiency in Zn, the tectum of the pollen also regained the structure observed in Zn sufficient plants. The wide lumen observed in Zn deficient pollen grains changed to pentagonal and hexagonal cells giving

Table 1 : Effect of Zn deficiency on diameter, pore size, exine thickness and viability of pollen grains of *Vigna radiata* L. cv-*T44* (Mean± SE of 20 observations)

Zn Supply (mg L-[1])	Pollen diameter (mm)	Pore diameter (mm)	Exine thickness (mm)	Pollen viability (%)
0.065	40.04±0.10	±5.44±0.11	3. 12±0.07	71±2
0.0065	36.68±0.07	4.56±0.05	3.41±0.04	44±2
0.0065+ 0.065	39.05±0.05	4.75±0.06	3.28.±0.08	55±2

the reticulum a more or less uniform pattern (Figure 1E). On receiving Zn sufficient solution, the muri of plants initially subjected to Zn deficieny became less sinous and lobed but they still retained the wax deposition on the sexinous elements of the lumen (Figure 1F).

DISCUSSION

If Zn supply is inadequate, emergence of flowers in green gram (*Vigna radiata* L. *cv T- 44*) is delayed and pollen grains of these plants undergo changes in ultrastructure. The pollen grains of Zn deficient plants have thicker exine with widely spaced irregular muri having particulate wax deposition in the lumen. In the present study, when Zn was supplied after the induction of Zn deficiency but prior to flowering, it was observed that supply of Zn to the Zn deficient plants partially restored the changes in exine structure induced by Zn deficiency and this was associated with increase in viability of pollen grains. These observations suggest a role of Zn in pollen development. Sharma *et al.* (1990) reported a critical requirement of zinc during microsporogenesis. Changes in the ornamentation of the exine structure and the stigmatic papillae leading to poor fertility was also observed earlier in faba beans (Pandey *et al.* 1995). It has been observed that control of exine pattern is mediated through moieties like RNA and mature pollen grains are rich in mRNA and enzymes (Willing *et al.* 1988; Wetzel & Jenson 1992). Disruption of RNA metabolism observed in Zn deficient plants (Sharma *et al.* 1987) may contribute to sterility of pollen grains due to changes in exine structure.

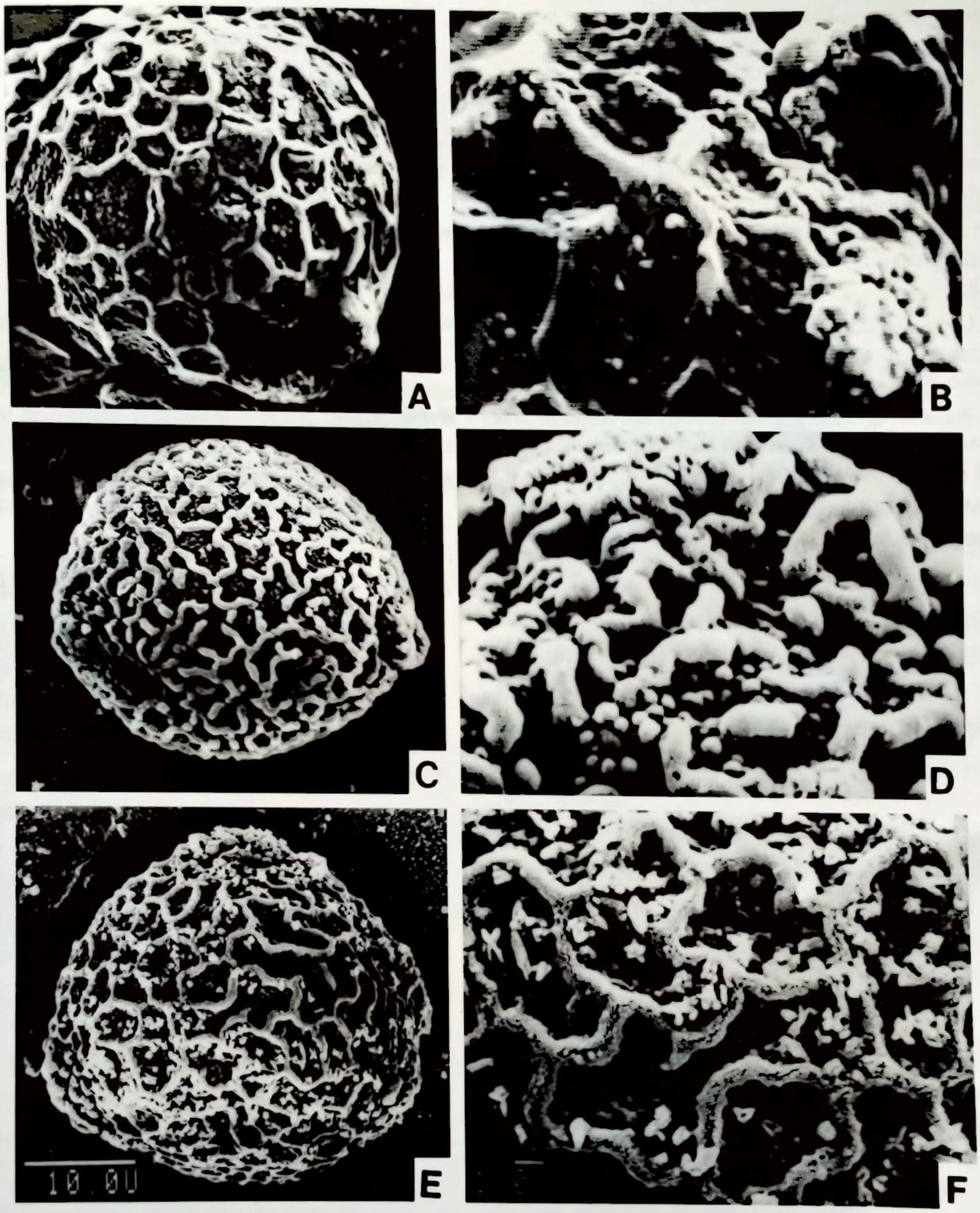

Fig. 1. A-F SEM of the pollen grains of *Vigna radiata* L. *cv T-44* grown with sufficient (A,B), deficient (C,D) and deficient given sufficient (E,F) zinc supply.

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