Indian J. Plant Physiol., Vol. 15, No. 4, (N.S.) pp. 338-342 (Oct.-Dec., 2010)

IMPROVING ZINC DENSITY AND SEED YIELD OF GREEN GRAM BY FOLIAR APPLICATION OF ZINC AT EARLY REPRODUCTIVE PHASE

G.C. PATHAK AND NALINI PANDEY*

Department of Botany, University of Lucknow, Lucknow-226007

Received on 10th Aug., 2010, Revised on 8th Dec., 2010

SUMMARY

The effect of foliar Zn treatments was studied on pollen-stigma interaction, its involvement in fertilization, seed Zn and seed yield. The plants grown with deficient supply of (ZnD) reduced the size of anthers, pollen producing capacity, size and viability of pollen grains. SEM studies of pollen grains showed the morphological changes in pollen shape and size with changes in the exine ornamentation. Flowers of ZnD plants showed a decrease in the pollen receptive area and a persistent cuticle over the stigmatic surface which affected the germinability of the pollen grains. The foliar applications of Zn to Zn deficient plants partially reversed the above effects. Foliar application of Zn also improved the yield, boldness, vigor and viability of seeds. Seed Zn was also appreciably enhanced in Zn sufficient plants given foliar Zn.

Key words: **Pollen grains, pollen producing capacity, reproductive phase, *Vigna radiata* (L.) Wilczek, Zn-density**

Zinc is an essential micronutrient for the normal healthy growth and reproduction of plants and animals. Zinc is the most common deficient micronutrient in soil in the world and almost 50% soils of India are deficient in Zn. These Zn deficient soils are under intensive cultivation of food crops, chiefly the cereals and legumes. Crop yield is reduced and the quality of crop products is frequently impaired when the supply of plant-available Zn is inadequate.

Mineral fertilization is one of the most important factors for improving yield. The crop yield and their quality can be improved by adequate soil and crop management practices. Since fertilization practices by Zn are very poor, not only the yield but also the Zn content in the seeds are highly reduced (Cakmak 2009). The latter has resulted in increasing incidences of malnutrition and health problems in children (Hotz and Brown 2004). Legumes are the major source of protein for the vegetarian population of the country and therefore it is important that these are intensively fortified with Zn to overcome these health problems.

Zinc nutrient status of plants also plays an important role in plant reproduction. Its deficiency inhibits different stages of plant reproductive development such as initiation of flowering, floral development, male and female gametogenesis, fertilization and seed development. Zinc is a constituent of a number of Zn finger proteins which forms a structural motif of DNA-binding region of the transcriptional regulatory proteins (Gamsjaeger *et al*. 2007) and play an important role in reproductive development. The physiological effects of Zn deficiency in pollen function, fertilization and reproductive development of plants was recently reported by Pandey *et al.* (2006, 2009). Swietlik (2002) reported that Zn deficiency corrected by foliar Zn spray prior to anthesis is very beneficial in terms of fruit yield.

*Corresponding author: E-mail: nalini_pandey@rediffmail.com

Fageria *et al.* (2009) found foliar fertilization of plants extremely beneficial for reproductive yield. In the present study we explored the effect of foliar application of Zn on reproductive development of green gram and its effect in enhancing not only the yield but also the Zn content for improved dietary intake by humans.

Plants of *Vigna radiata* (L.) Wilczek var. IPM-99-125 Meha were grown in sand culture under glass house in controlled conditions. During the course of the experiment the photoperiod was 12 h with maximum light intensity PPFD at 12.00 noon ranging between 1000 and 1200 µmol. The temperature during 24 h ranged between 35 and 42^{0}C (maximum) and 26 and 30^{0}C (minimum). The humidity at 9.30 A.M. was 78% and 98%. The composition of the nutrient solution supplied was: 4 mM Ca $(NO_3)_2$, 4 mM KNO_3, 2 mM $MgSO_4$ 1.33 mM NaH_2PO_4, 0.33 mM H_3BO_3, 0.1 mM Fe-EDTA, 10 µM $MnSO_4$, 1 µM $CuSO_4$, 0.2 µM Na_2MoO_4, 0.1 mM NaCl, 0.1 µM $CoSO_4$, and 0.1 µM $NiSO_4$. Zinc was supplied as 0.2 µM (deficient-D) and 1 µM Zn (sufficient-S) in the form of $ZnSO_4$. Zinc deficient (ZnD) and Zn sufficient (ZnS) plants were given foliar spray of 0.1% $ZnSO_4$ at the initiation of flowering (at 55 d) and these treatments were referred to as deficient foliar (ZnDF) and sufficient foliar (ZnSF) respectively. Plants receiving the four treatments- Zn deficient (ZnD), sufficient (ZnS), deficient foliar (ZnDF) and sufficient foliar (ZnSF) were grown to maturity and quantified for different parameters. Dry matter yield was taken after 15 days of Zn treatment by oven drying the plant samples. Seed yield was determined at maturity (95 d). Leaf and seed tissue Zn was determined by atomic absorption spectrophotometer after wet acid digestion (HNO_3: $HClO_4$; 10:1) of oven dried (80^{0}C) materials.

To observe the stigmatic secretion and surface details of stigma and pollen grains, both fresh and fixed samples were examined. Fresh flowers were collected between 0700 to 0900 h and fixed in 1.5 % glutaraldehyde and 0.05 M phosphate buffer at pH 7.2 for 14 h with 2 h post fixation in 1% osmium tetraoxide. For SEM, the pollen grains and stigma were dehydrated through graded ethanol-isoamyl series and dried in critical point dryer (CPD), mounted on stubs and coated with gold palladium. Specimens were observed and photographed by a LEO 430 Scanning Electron Microscope (LEO Electron Microscopy Ltd. Cambridge UK). Light microscopic examination of the pollen grains and anthers was done under a Nikon E- 400 microscope and photographed by a Nikon F 70 camera. Size of anther and pollen grains were measured after mounting the preparation in glycerine jelly.

The pollen germination was determined by germinating the pollen grains in a culture medium by the hanging drop method in a cavity slide (Brewbaker and Kwack, 1963). Scoring was based on 5 sets of 20 pollen grains from flowers from each treatment, under the microscope. The pollen grains having pollen tubes longer than the pollen diameter were taken as viable. Each treatment had three replicates and experimental data were analyzed statistically using ANOVA. The mean values and the least significant difference (LSD at P= 0.05) are presented in the table.

The effect of variation in Zn supply was reflected as differences in plant growth 30 days after supply. About this time height, branching and leaf size of plants grown with 0.2 µM Zn supply appeared restricted and their young trifoliates showed interveinal chlorosis. The leaves developed marginal chlorosis of leaflets leading to necrosis. The difference in growth was observed throughout growth period. The leaves and seed of Zn deficient plants had lower concentration of Zn but foliar application of Zn increased Zn concentration up to 2 to 3 times (Table 1 and 2).

Zinc deficiency stress not only retarded vegetative growth and produced visible symptoms of Zn deficiency but also affected the reproductive development. The flowering was delayed by almost a week and very few flowers were developed in plants which were grown with low Zn supply (0.2 µM Zn). The Zn deficient plants showed a significant reduction in anther size and PPC which increased by foliar supplements of Zn to the deficient plants but did not show much difference in Zn sufficient plants (Table 1). SEM studies of pollen grains showed the morphological changes in pollen shape and size. The pollen grains of ZnD plants showed changes in the exine ornamentation with waxy deposition on muri and irregular reticulations (Fig. 1A) as compared to the ZnS plants (Fig 1B). Pollen staining by acetocarmine was taken as the count for pollen fertility. A large number

Table 1. Effect of Zn treatment on dry matter yield, leaf tissue Zn concentration, flower number, anther, pollen size and pollen viability of green gram (*Vigna radiata* (L.) Wilczek). (ZnD = Zinc deficient; ZnDF = ZnD given foliar Zn; ZnS = Zinc sufficient; ZnSF = ZnS given foliar Zn).

Parameters	Zn Treatments				L.S.D
	ZnD	ZnDF	ZnS	ZnSF	(P=0.05)
Dry matter yield (g plant^{-1})	4.32 ± 0.35	6.45 ± 0.46	10.15 ± 0.52	11.92 ± 0.39	1.32
Leaf tissue Zn (µg g^{-1} dry wt)	8.7 ± 0.24	15.2 ± 0.32	30.70 ± 0.67	48.2 ± 0.74	3.92
No. of flower plant^{-1}	20 ± 0.45	22 ± 0.62	24 ± 0.56	24 ± 0.73	1.65
Anther size (µM)	324 ± 10.32	398 ± 13.36	465 ± 20.38	470 ± 18.32	28
Pollen size (µM)	62.5 ± 3.35	69.2 ± 5.42	75.0 ± 4.62	75.0 ± 5.44	3.88
Pollen viability (% germination)	38 ± 1.32	70 ± 2.32	80 ± 3.38	85± 4.37	3.40

Table 2. Effect of Zn treatment on pod and seed number, pod and seed weight, seed viability and Zn concentration of seed in green gram (*Vigna radiata* (L.) Wilczek). (ZnD = Zinc deficient; ZnDF = ZnD given foliar Zn; ZnS = Zinc sufficient; ZnSF = ZnS plants given foliar Zn).

Parameters	Zn Treatments				L.S.D
	ZnD	ZnDF	ZnS	ZnSF	(P=0.05)
No. of pod plant^{-1}	11 ± 0.33	15 ± 0.52	19 ± 0.39	20 ± 0.67	1.5
g. dry wt. of pod plant^{-1}	2.10 ± 0.23	4.86 ± 0.34	6.30 ± 0.43	7.45 ± 0.37	0.56
g. dry wt. of 100 pod	15.63 ±0.36	18.67 ±0.29	29.89 ± 0.32	30.06± 0.37	1.95
No. of seed plant^{-1}	15 ± 0.28	20 ± 0.92	25 ± 0.74	27 ± 0.63	1.8
g. dry wt. of seed plant^{-1}	1.73 ± 0.03	3.32 ± 0.12	5.17 ± 0.28	5.58 ± 0.25	0.45
g. dry wt. of 100 seed	10.56 ±0.30	15.95 ±0.26	20.68± 0.36	21.56± 0.62	1.62
Seed viability (% germination)	55 ± 2.34	86 ± 3.20	90 ± 2.72	92 ± 4.31	6.68
Seed tissue Zn (µg g^{-1}dry wt.)	12.60± 0.39	40.90 ±0.83	52.80 ± 0.92	60.20± 0.78	4.62

of pollen grains of ZnD plants failed to stain with acetocarmine (Fig 1C) and showed significant decreases *in vitro* germination (Table 1) as compared to the ZnS plants (Fig. 1D). The pollen viability was however increased in ZnDF and ZnSF plants significantly, more so in the former (Table 1). A significant decrease *in vitro* germination by the Zn deficient pollen grains and subsequent increase after Zn application suggests loss of viability due to low Zn. Franklin-Tong (1999) and Zinkl *et al.* (1999) have shown that alterations in pollen coat waxes and lipids may lead to loss of pollen fertility. This is corroborated by the observed changes in the exine architecture of ZnD pollen grains.

Scanning electron microscopy of stigmas from flowers of ZnD plants showed a decrease in the pollen receptive area and a persistent cuticle over the stigmatic surface (Fig. 1E). The stigmatic head of ZnS plants showed a ruptured stigmatic cuticle and heavy exudation with a large number of pollen grains adhering to them (Fig. 1F). Rupture of the cuticle over the stigmatic papillae is crucial to adhesion of pollen grains on the

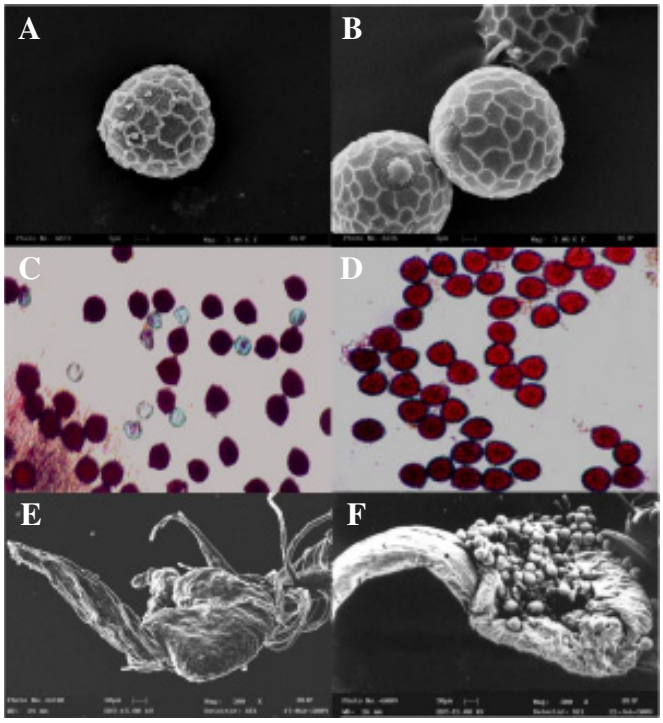

Fig. 1. Scanning electron micrographs of Zn deficient (A) and Zn sufficient (B) pollen grain. Pollen viability in Zn deficient (C) and Zn sufficient (D) plants. SEM of Zn deficient (E) and Zn sufficient (F) stigmas of green gram (*Vigna radiata* L).

stigma (Heslop-Harrison 2000). The rupture of the stigmatic cuticle involves the activities of certain enzymes such as cutinases (Hiscock *et al*. 1994, Edlund *et al.* 2004) and esterases (Dafni and Maues 1998, Hiscock *et al*. 2002) which provide a conducive environment for facilitating the germination of pollen grains leading to fertilization and seed set. Thus poor adhesion of pollen grains and growth of pollen tube on the stigmatic head due to intact cuticle and poor stigmatic exudation caused limited fertilization and poor seed setting in Zn deficient green gram plants.

Zinc deficient plants failed to produce pod and seed in plants grown with low Zn supply (0.2 µM Zn). In comparisons with the plants given low Zn supply the number and weight of pods and seeds formed was high in plants given ZnS and ZnSF supply (Table 2). Zinc deficiency not only affected the seed setting but it also affected their viability (Table 2). Foliar supplementation of 0.1% $ZnSO_4$ to ZnD and ZnS plants increased the seed yield by 37 % and by 8 % in ZnDF and ZnSF plants respectively. The Zn concentration in seeds of ZnD plants was 12.60 and 40.90 µg g^{-1}dry wt. in ZnDF plants. In ZnS plants Zn concentration in seeds increased from 52.80 to 60.20 µg g^{-1}dry wt in ZnSF plants.

Foliar application of Zn to ZnD at the initiation of flowering partially reversed the adverse effect of Zn deficiency on the seeds and their germination. These changes are suggestive of a role of Zn in seed development and maturation. Foliar application of $ZnSO_4$ at the time of initiation of flowering to Zn deficient plants minimized the Zn deficiency effect but foliar application of Zn to Zn sufficient plants made little differences to number of flower and seeds. In the present study we observed that the foliar application of Zn improves seed Zn concentration up to 2 to 3 times in ZnD and in ZnS plants. We concluded that loss of pollen function and impairment in fertilization lead to poor development of seed and contribute to poor seed yield of legumes grown on low Zn soils. This loss can be compensated through Zn fertilization of crops at the onset of reproductive phase. Judicious application of foliar Zn under Zn deficiency and sufficient conditions can also be used as a strategy to increase the Zn density in seeds for improved human consumption.

ACKNOLEDGEMENTS

The author Girish Chandra Pathak is grateful to the Department of Science and Technology, New Delhi, for financial assistance under Project No. SR/FT/L-76/2006. The authors are grateful to Director, BSIP, Lucknow for providing SEM facility.

REFERENCES

Brewbaker, J.L. and Kwack, B.H. (1963). The essential role of calcium ion in pollen germination and pollen tube growth. *American J. Bot.* **50:** 859-865.

Cakmak, I. (2009). Enrichment of Fertilizers with Zinc: An Excellent Investment for Humanity and Crop Production in India. *J. Trace Element Medicine Biol.* **23**: 281-289.

Dafni, A. and Maues, M.M.(1998). A rapid and simple procedure to determine stigma receptivity. *Sex. Plant Repr.* **11:** 177–180.

Edlund, A.F., Swanson, R. and Preuss, D. (2004). Pollen and stigma structure and function: The role of diversity in pollination. *Plant Cell (Suppl)* **16:** S84-S97.

Fageria, N.K., Barbosa-Filho, M.P., Moreira, A. and Guimaraes, C.M. (2009). Foliar fertilization of crop plants. *J. Plant Nutr.* **32**: 1044-1064.

Franklin-Tong, V.E. (1999). Signaling and the modulation of pollen tube growth. *Plant Cell* **11:** 727-738.

Gamsjaeger, R., Liew, C.K., Loughlin, F.E., Crossley, M. and Mackay J.P. (2007). Sticky fingers: zinc-fingers as protein-recognition motifs. *Trends Biochem. Sci.* **32:** 63-70.

Heslop-Harrison, Y. (2000). Control gates and micro-ecology: The pollen–stigma interaction in perspective. *Ann. Bot.* **85:** 5-13.

Hiscock, S.J., Bown, D., Gurr, S.J. and Dickinson, H.G. (2002). Serine esterases are required for pollen tube penetration of the stigma in Brassica. *Sex. Plant Repr.* **15:** 65–74.

Hiscock, S.J., Dewey, F.M., Coleman, J.O.D. and Dickinson, H.G. (1994). Identification and localization of an active cutinase in the pollen of *Brassica napus* L. *Planta* **193:** 377–384.

Hotz, C., and Brown, K.H. (2004). Assessment of the Risk of Zinc Deficiency in Populations and Options for its Control. *Food Nutr. Bull.* **25:** 94-204.

Pandey, N., Pathak, G.C. and Sharma, C.P. (2009). Impairment in reproductive development is a major factor limiting seed yield of black gram under zinc deficiency. *Biol. Plant.* **53:** 723-727.

Pandey, N., Pathak, G.C., Sharma, C.P. (2006). Zinc is critically required for pollen function and fertilization in lentil. *J. Trace Elem. Med. Biol.* **20:** 89-96,

Swietlik, D. (2002). Productivity, growth and leaf mineral composition of orange and grape fruit trees, foliar sprayed with zinc and manganese. *J. Plant Nutr.* **14**: 129-142.

Zinkl, G.M., Zwiebel, B.I., Grier, D.G. and Preuss, D. (1999). Pollen-stigma adhesion in *Arabidopsis:* a species-specific interaction mediated by lipophilic molecules in the pollen exine. *Development* **128:** 5431-5440.