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Foliar application of Zn at flowering stage improves plant's performance, yield and yield attributes of black gram

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Black gram plants subjected to varying levels of Zn supply (0.01 to 10 µM Zn) showed optimum growth and dry matter yield in plants receiving 1 µM Zn. The dry matter yield of plants decreased in plants receiving 0.01 and 0.1 µM Zn (deficient) and excess levels of Zn (2 and 10 µM Zn). The plants grown with Zn deficient supply showed delayed flowering, premature bud abscission, reduced size of anthers, pollen producing capacity, pollen viability and stigma receptivity resulting in poor pod formation and seed yield. Providing Zn as a foliar spray at pre-flowering stage minimized the severity of Zn deficiency on reproductive structure development and enhanced the seed nutritional status by enhancing seed Zn density, seed carbohydrate (sugar and starch content) and storage proteins (albumins, globulins, glutenins, and prolamines).

Keywords: Biofortification, Black gram, Foliar spray, Seed Zn, Seed yield

Reproduction is one of the most important events during the life cycle of higher plants. It is a multistep process which starts from floral primordial initiation and ends at seed maturity. Several environmental factors like drought[1], salinity[2] and micronutrient stress[3] affect the normal process of reproduction. Among micronutrient stress that limits reproduction, Zn deficiency figures predominantly. Plants exposed to Zn deficiency show delayed flowering, premature bud abscission, reduced seed set and seed yield with low Zn content. This may be attributed to poor pollen fertility[4] and impaired pollen stigma interaction[5,6]. The role of Zn in sexual reproduction may also be due to transcription factors like *ID1*, *TAZ1*[7], *PhZPT-3-3* and *PhZPT-2-10* and *DBB1a*[8] which are Zn-finger motifs, and are known to be involved in activating target genes which are specifically expressed in particular floral organs during flowering/reproduction.

Zinc deficiency is one of the most common deficiencies prevalent in the world[9]. The Indian soil is Zn deficient due to intensive cultivation of food crops, chiefly the cereals and legumes, which limits the yield and Zn content in the seeds. Zinc deficiency in human beings impairs a number of metabolic functions. Zn has a key role in alleviating nutritional disorders and malnutrition that exist in much of the developing world including India, causing health problems especially in children. Therefore increasing seed Zn concentration is a global demand to compensate for Zn-deficiency-related health problems in human beings caused by low dietary Zn intake. Foliar spray is a short term approach not only for improving yield in deficient soils but also to achieve nutritional efficacy[10,11]. Since legumes are the major source of protein for the vegetarian population of the country it is important that these are intensively fortified with Zn to overcome health problem. Black gram is an important legume grown in India and is an important source of dietary protein and mineral nutrient. However productivity of black gram like other legumes has been low probably due to widespread deficiency of Zn.

The present communication reports the changes and modifications in floral characters, reproductive development and yield in black gram by using Zn nutrition as foliar application. The study also reports the effects of Zn application on quality of seed in terms of the status of various metabolites.

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Materials and Methods

Plant material and experimental design—Plants of black gram (*Vigna mungo* cv. DPU-88-31) were grown in purified sand with nutrient solution containing 4 mM KNO_3, 4 mM $Ca(NO_3)_2$, 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 $CuSO_4$, 1 µM $ZnSO_4$, 0.1 µM Na_2MoO_4, 0.1 µM NaCl, 0.1 µM $CoSO_4$ and 0.1 µM $NiSO_4$ at normal (1.0 µM) and deficient level (0.1 µM) of Zn supply[12]. Zinc was supplied as $ZnSO_4$ and the deficient (0.01 µ*M*) and normal level of Zn supply (1.0 µ*M*) was chosen from a preliminary experiment conducted to study the effect of varying level of Zn supply ranging from deficiency to excess (0.01, 0.1, 1.0, 2.0 and 10 µ*M* Zn) on growth and yield of black gram.

After seedling emergence pots were separated in two sets. The Ist set was supplied with normal (1.0 µ*M* Zn) and 2nd set was supplied with deficient Zn (0.01 µ*M* Zn). Prior to flowering, the Zn deficient plants were further divided into four sets. While one lot of Zn deficient plants continued to receive deficient supply, the other sets of Zn deficient plants were sprayed with 0.1% $ZnSO_4$ at three different stages of reproductive development i.e. prior to flowering (at vegetative stage), at initiation of bud formation and prior to anthesis. The foliar spray was done thrice at an interval of 4 days on all leaves and the bases of the plants were covered so that solution did not trickle to the pots. The total treatments were as follows:

Ist set: Zn sufficient plants (1.0 µ*M* Zn)	Control
2nd set: Zn deficient plants without any foliar spray (0.01 µ*M* Zn)	ZnD
3rd set: ZnD plants given foliar spray of 0.1% $ZnSO_4$ before flowering	Zn+F1
4th set: ZnD plants given foliar spray of 0.1% $ZnSO_4$ at initiation of bud formation	Zn+F2
5th set: ZnD plants given foliar spray of 0.1% $ZnSO_4$ prior to anthesis	Zn+F3

During experimentation, light (PAR) ranged between 161 to 217 Wm^{-2} at 12.00 hrs, relative humidity (RH) ranged between 68-98% at 09.30 hrs. and minimum and maximum temperature ranged between 24-30°C and 30-42°C respectively.

Dry matter yield and concentration of Zn was determined in leaves, stem, root and seeds in wet acid digests (HNO_3: $HClO_4$) by atomic absorption spectrophotometer (Perkin Elmer Analyst 300). The concentration of Fe, Mn and Cu was also determined in the leaves.

Pollen-stigma interaction—During anthesis flowers were fixed in 1.5% glutaraldehyde and 0.05 *M* phosphate buffer (*p*H 7.2) for 2 h, washed in phosphate buffer and postfixed for 2 h in 1% (w/v) osmium tetroxide in the phosphate buffer at 4 °C. The pollen grains were acetolysed[13] and samples were dehydrated through an ethanol-isoamyl series and dried in critical point dryer (CPD), mounted on stubs and coated with gold palladium. Photographs were taken with a LEO 430 scanning electron microscope (LEO Electron Microscopy Ltd. Cambridge U.K.). Light microscope (LM) examination of the anthers and pollen grains was done under a Nikon E-400 microscope.

Pollen producing capacity (PPC) per anther was determined by gently crushing anther in 10% (v/v) glycerol. The total number of pollen grains was counted under microscope. The pollen viability was determined by germinating pollen grains by hanging drop method in solution culture medium containing (%) sucrose (10), boric acid (0.01), calcium nitrate (0.03), magnesium sulphate (0.02) and potassium nitrate (0.01)[14]. Scoring was done of 10 sets of 20 flowers each, from each treatment.

Cytochemical localization of enzymes i.e. peroxidase (POD), acid phosphatase (APase) and esterase (Est) on the stigmatic surface were determined[5]. The stigma were stained for POD for 30 min in 0.5% paraphenylenediamine and 0.5% H_2O_2 and washed thoroughly in phosphate buffer before mounting. For localization of APase the stigma were placed for 15 min in a reaction mixture containing α-naphthyl phosphate (Sigma) as the substrate, fast garnet GBC (Sigma) as the coupling agent in 0.1 *M* acetate buffer (*p*H 4.0) and 10% $MgCl_2$. Staining for localization of esterase was done in a freshly prepared solution of α-naphthyl acetate (Sigma) in 0.15 *M* phosphate buffer, 10% sucrose and 25 mg Fast Blue B salt (Sigma). For control α-naphthyl acetate was excluded from the reaction mixture.

Seed yield and quality—Ten plants per treatment in triplicates were tagged prior to foliar application and the number of pod formed thereafter were counted and recorded. When the pods matured, the number, length and weight of pods and seeds formed were also measured. Seed viability was determined in 50 seeds

per treatment in triplicates, placed in petridishes lined with water-moist filter paper and germination was counted after 3 days.

At 105 day, mature and dried seeds were crushed and fixed in 50% (v/v) boiling ethanol (1:10) and ground at room temperature for determination of reducing and non-reducing sugars[15] and starch[16]. Seed storage proteins were extracted after removing seed coat[17]. The seeds were ground to a dry powder and then extracted in acetone and centrifuged at 11,500 *g*. The residue was air dried and the seed flour was extracted with water for albumins, 5% NaCl for globulins, 0.1 N NaOH for glutenins and 70% ethanol with 2 drop of mercaptoethanol for prolamines. Each of the extracts was again centrifuged and supernatant was taken for protein estimation. The protein in the above extracts was estimated by the method of Lowry[18].

Statistical analysis—The data were statistically evaluated by ANOVA. The results are presented as means ± SD from 3 observations Significant differences ($P \leq 0.05$) between means were also determined.

Results

Zinc deficient black gram plants showed a marked reduction in vegetative growth, condensation of internodes, suppression of branching and reduction in leaf size. The leaves also developed visible symptoms such as marginal chlorosis which was partially recovered by foliar application of Zn. A dose response effect was observed on the growth and dry matter yield of black gram plants (Table 1) subjected to varying levels of Zn supply (0.01 to 10 μ*M* Zn). Optimum dry matter yield was obtained in plants receiving 1 μ*M* Zn. The optimum yield observed at 1 μ*M* Zn supply was also reflected in the floral analysis (flower numbers, anther and pollen size, pollen viability) pod and seed yield (Table 1) which increased with increase in Zn supply from 0.01 to 1 μ*M* Zn and then decreased at the excess levels (2 and 10 μ*M* Zn). Optimum level of Zn supply for black gram was 1 μ*M* Zn and this level was treated as control for the experiment conducted to study the effect of foliar application of Zn on yield of black gram plants.

The dry matter yield of roots, stem and leaves was decreased in ZnD plants but increased significantly with foliar Zn supply. However the recovery in dry weight (DW) of leaves and roots was not above the control plants (1 μ*M* Zn). As compared to control, the foliar Zn application increased total DW and treatment Zn+F1 presented the best results (Table 2A).

Tissue Zn concentration of ZnD plants was drastically reduced but increased in plants given foliar Zn. The increase in Zn concentration in stem and roots was less than the control, but Zn concentration in the leaves and seeds of plants given foliar Zn was much more than control being highest in Zn+F2 treatment (Table 2B). The tissue status of the

Table 1—Effect of Zn supply on the dry matter yield, floral analysis and reproductive yield of black gram (*Vigna mungo* L var. DPU-88-31).
[Values are mean ±SE from 3 experiments.]

Parameters	Zn supply (μM)				
	0.01	0.1	1.0	2.0	10.0
Dry matter yield : g plant^{-1}					
Leaves	^{c}0.660±0.04	^{b}0.779±0.07	^{a}0.970±0.05	^{a}1.053±0.07	^{a}0.952±0.09
Stem	^{d}0.380±0.03	^{c}0.590±0.04	^{a}1.585±0.06	^{b}1.068±0.08	^{b}0.992±0.08
Root	^{d}0.063±0.02	^{c}0.090±0.05	^{a}0.195±0.08	^{a}0.184±0.07	^{b}0.146±0.03
Whole plants	^{e}1.103±0.13	^{d}1.159±0.15	^{a}2.750±0.09	^{a}2.305±0.14	^{c}2.090±0.17
Flower No.	^{d}16.0 ±1.23	^{c}23.0±1.36	^{a}44.0±2.56	^{b}34.0±2.52	^{b}33.0±2.41
Anther size l×b (μm)	^{e}423±21.29	^{d}473±24.43	^{a}790±31.23	^{b}758±41.26	^{b}718±29.53
Pollen size (μm)	^{c}58.1±4.10	^{b}76.3±7.09	^{c}91.2±5.78	^{a}88.5±4.89	^{a}84.7±6.22
Pollen viability (%)	^{d}32±2.77	^{c}47±3.68	^{a}90±5.28	^{a}84±5.30	^{a,b}80±6.70
No. of pods plant^{-1}	^{d}8±0.63	^{c}11±0.80	^{a}25±1.28	^{a}25±1.24	^{b}20±1.33
Pod wt. plant^{-1} (g)	^{c}0.099±0.03	^{b}0.135±0.05	^{a}0.256±0.22	^{d}0.214±0.23	^{a}0.184±0.13
No. of seeds plant^{-1}	^{d}44±2.03	^{c}68±4.26	^{e}145±10.63	^{a}120±13.26	^{b}102±11.24
Seed wt. plant^{-1} (g)	^{b}1.6±0.02	^{c}1.9±0.04	^{a}3.7±0.18	^{a}3.2±0.08	^{b}2.9±0.14

Differences between means with different letters in the same row are significant at $P \leq 0.05$.

Table 2—Effect of foliar Zn supply on dry matter yield (A), tissue and seed Zn (B) and leaf Fe, Mn and Cu (C) of black gram plants (*Vigna mungo* L. DPU-88-31).
[Values are mean ±SE from 3 observations]

Plant parts	Zn supply				
	Control	ZnD	Zn+F1	Zn+F2	Zn+F3
	(A) DW: g plant^{-1}				
Root	[a] 0.195±0.002	[d] 0.058±0.0011	[b] 0.109±0.001	[b] 0.109±0.011	[c] 0.069±0.011
Stem	[a] 1.54±0.011	[e] 0.40±0.009	[a] 1.63±0.012	[c] 1.27±0.022	[d] 0.96±0.017
Leaves	[a] 1.00±0.019	[c] 0.63±0.012	[b] 0.92±0.033	[c] 0.73±0.033	[c] 0.68±0.022
Whole plant	[a] 2.735±0.081	[e] 1.061±0.061	[b] 2.659±0.041	[c] 2.109±0.059	[d] 1.709±0.065
	(B) Tissue Zn: µg g^{-1} DW				
Root	[a] 23.96±0.026	[b] 9.64±0.033	[d] 15.40±0.036	[b] 19.20±0.043	[c] 17.40±0.073
Stem	[a] 28.36±1.94	[c] 10.53±1.63	[b] 23.20±3.81	[b] 28.01±2.59	[b] 25.17±2.75
Leaves	[a] 42.68±1.51	[d] 11.33±1.24	[b] 53.90±1.49	[c] 46.90±1.56	[c] 46.80±1.49
Seed	[a] 33.0±1.09	[d] 18.36±0.99	[a] 32.67±1.06	[b]39.28±1.01	[b] 36.30±1.05
	(C) Tissue Leaf Fe, Mn, Cu: µg g^{-1} DW				
Fe	[a] 113±10.34	[a] 115±14.53	[a] 129±10.89	[a,b]118±12.45	[a] 121±9.85
Mn	[b] 80.9±11.51	[a] 90±12.45	[a] 97±11.68	[b] 84±10.56	[e] 91±11.23
Cu	[b] 10.92±1.04	[a] 11.15±1.09	[a] 12.76±0.78	[b] 10.56±0.89	[e] 12.34±1.32

(Control: 1.0 µM Zn; ZnD: 0.01 µM Zn. Foliar spray to ZnD plants: Zn+F1= 0.1% $ZnSO_4$ before flowering; Zn+F2= 0.1% $ZnSO_4$ initiation of bud formation; Zn+F3= 0.1% $ZnSO_4$ prior to anthesis)

Differences between means with different letters in the same row are significant at $P \leq 0.05$.

micronutrients like Fe, Mn and Cu was determined in the leaves of Zn deficient black gram plants which as compared to control contained normal to high values of these micronutrients (Table 2C).

Table 3 shows that Zn deficiency reduced the number of flowers formed and foliar application increased it. The flower size was also increased and was almost of the size of control plants after foliar supply of Zn. Zinc deficiency decreased the anther size, which was recovered by foliar application of Zn. Plants at Zn+F1 showed highest recovery in anther size as compared to other treatments but no significant difference was observed in respect to Zn+F2 treatment. The PPC also showed a significant decrease in the ZnD plants as compared to control. The PPC was increased by Zn supply and the increase was most significant in Zn+F2 plants. Zinc deficiency reduced pollen size by 16% from control plants whereas foliar Zn increased the pollen size to control values. Zinc deficiency increased exine thickness of the pollen grains by 27% more than the control pollen grains and the increase was reduced by resupply of Zn (Table 3). The pollen viability of the ZnD pollen grains was decreased but foliar Zn increased the viability which was as high as that observed in the control plants in Zn+F2 plants.

Compared to that of control, the pod of ZnD plants were smaller, hairy, shrunken and grey in color. The number of pods per plant increased in foliar Zn supplied plants to values more than control. The pod length of foliar sprayed plants was also significantly higher than control, and had more number of seed set per pod (Table 3). The pod weight per plant showed the same trend as the number of pods per plants and the highest increase was observed at Zn+F2 followed by Zn+F1 and Zn+F3 as compared to control.

The number and weight of seeds was increased by foliar Zn application at each stage. There was an increase in seed number in the order Zn+F2>Zn+F3>Zn+F1>control>ZnD (Table 3). The seed size was also significantly reduced by Zn deficiency. Seed size increased with foliar Zn application and was significantly highest at Zn+F2 level being more than control. At Zn+F2 level seed showed a significantly higher percentage of seed viability as compared with control and other treatments (Table 3).

Pollen grains from ZnD plants showed incomplete reticulation, thick and wider muri with waxy deposition (Fig.1a-b). *In vitro* germination revealed that pollen tube from control and foliar sprayed pollen grains grew longer and rarely showed bursting (Fig. 1c), while pollen tube from ZnD pollen grains did not grow and most of them showed burst tips (Fig. 1d). SEM of the stigmatic surface revealed large number of germinating pollen grains adhering to it

Table 3—Effect of foliar Zn supply on floral parameters of black gram plants (*Vigna mungo* L.DPU-88-31)
[Valuesare mean ±SE from 3 observations each]

Parameters	Zn supply				
	Control	ZnD	Zn+F1	Zn+F2	Zn+F3
Flower No.	[a] 46.0±1.04	[d] 20.0±2.06	[b] 38.0±1.09	[b] 40.0±2.23	[c] 34.0±1.98
Flower size (cm)	[a] 1.6±0.03	[b] 1.1±0.02	[a] 1.5±0.09	[a] 1.6±0.10	[a] 1.4±0.09
Anther size l×b (µm)	[b] 794±1.56	[d] 46±1.53	[a] 824±1.62	[a] 812±1.34	[c] 773±1.24
PPC (grain anther^{-1})	[b] 591±2.18	[e] 253±1.16	[c] 522±3.21	[a] 612±1.99	[d] 461±2.35
Pollen size (µm)	[a] 89.4±2.02	[c] 76.1±2.09	[b] 86.4±2.06	[b] 88.2±2.84	[b] 86.3±2.35
Exine thickness (µm)	[b] 3.3±0.24	[a] 4.2±0.12	[b] 3.4±0.35	[b] 3.4±0.23	[b] 3.5±0.26
Pollen viability (%)	[a] 92±1.89	[c] 49±1.34	[b]81±2.00	[a] 92±2.31	[b] 79±2.56
Pod no. plant^{-1}	[c] 23±1.80	[d] 10±1.33	[a] 30±1.56	[a] 33±1.79	[b] 28±1.16
Pod length (cm)	[a] 4.01±0.20	[c] 2.45±0.53	[a] 4.20±0.44	[a] 4.23±0.23	[a] 4.21±0.39
Pod wt. plant^{-1} (g)	[c] 0.267±0.012	[d] 0.156±0.011	[b] 0.307±0.019	[a] 0.319±0.021	[b] 0.305±0.013
Seed no. plant^{-1}	[d] 142±2.44	[e] 46±1.53	[c] 180±1.71	[a] 198±2.79	[b] 185±1.53
Seed size (mm)	[b] 0.33±0.004	[c] 0.14±0.003	[b] 0.35±0.001	[a] 0.40±0.006	[b] 0.37±0.004
Seed wt. plant^{-1} (g)	[b] 3.8±0.041	[c] 1.7±0.033	[b] 4.0±0.023	[a] 4.5±0.028	[b] 4.2±0.019
Seed viability (%)	[a] 91±2.36	[d] 42±2.02	[c] 84±1.03	[a] 95±1.14	[b] 89±2.17

(Control: 1.0 µM Zn; ZnD: 0.01 µM Zn; foliar spray to ZnD plants: Zn+F1= 0.1% $ZnSO_4$ before flowering, Zn+F2= 0.1% $ZnSO_4$ before initiation of bud formation, Zn+F3= 0.1% $ZnSO_4$ prior to anthesis).
Differences between means with different letters in the same row are significant at $P \leq 0.05$.

(Fig. 1e) whereas ZnD plants showed few but empty pollen grains on the stigma (Fig. 1f). As compared to control plants, activity of esterase in the stigmatic surface was decreased in the Zn deficient plant (Fig. 1g, h) and that of peroxidase and acid phosphatase was increased (Fig. 1g-l).

Zinc deficiency decreased the reducing, non-reducing sugars, total sugars and starch concentration (Fig. 2a-d) in seeds, whereas the foliar application of Zn at Zn+F2, showed highest increase in total sugars followed by Zn+F3 and Zn+F1.

The seed storage protein concentration was decreased at ZnD and increased with foliar application of Zn. The increase in storage proteins, globulins, albumins, prolamins and glutenins were highest at Zn+F2 level, followed by Zn+F1 and Zn+F3 levels (Fig. 2e-h) but later two were more in control.

Discussion

Black gram plants deficient in Zn showed reduction in growth and produced typical symptoms like reduction in leaf size, shortening of internodes and chlorosis in leaves which were specific to Zn deficiency, as reported earlier[6,19]. Zinc deficiency decreased DW of plants which was increased by foliar spray of Zn and defines its need for growth in the highly metabolized meristematic regions where adequate Zn interacts with auxin to promote plant growth[20].

The concentration of Zn in leaves, stem and roots was decreased by Zn deficiency but foliar application significantly increased the Zn concentration especially in the stem and leaves. Similarly, seed Zn concentration increased at all the three stages of foliar spray as compared to control plants which showed that foliar applied Zn ions possess high mobility within the plants. This increase in seed Zn concentration further supported the yield parameters like seed set per pod, weight of seeds per pod and seed quality in terms of seed carbohydrates and seed protein concentration. The tissue status of the micronutrients like Fe, Mn and Cu did not indicate any deficiency and ruled out the possibility of the effect of their deficiencies in the reproductive development of black gram[3].

Floral analysis of black gram grown with deficient Zn supply showed reduction in size of anthers formed which maybe due to aberrant anther wall (tapetum) development[7]. Decrease in PPC could also result due to Zn deficiency induced changes in the structure and functions of tapetum by virtue of role of Zn as structural motifs of the Zn-finger protein and their role in anther development[21]. SEM studies revealed that waxy deposition filled the cavities between the baculae in Zn deficient pollen grains which creates a hydrophobic environment injurious to pollen

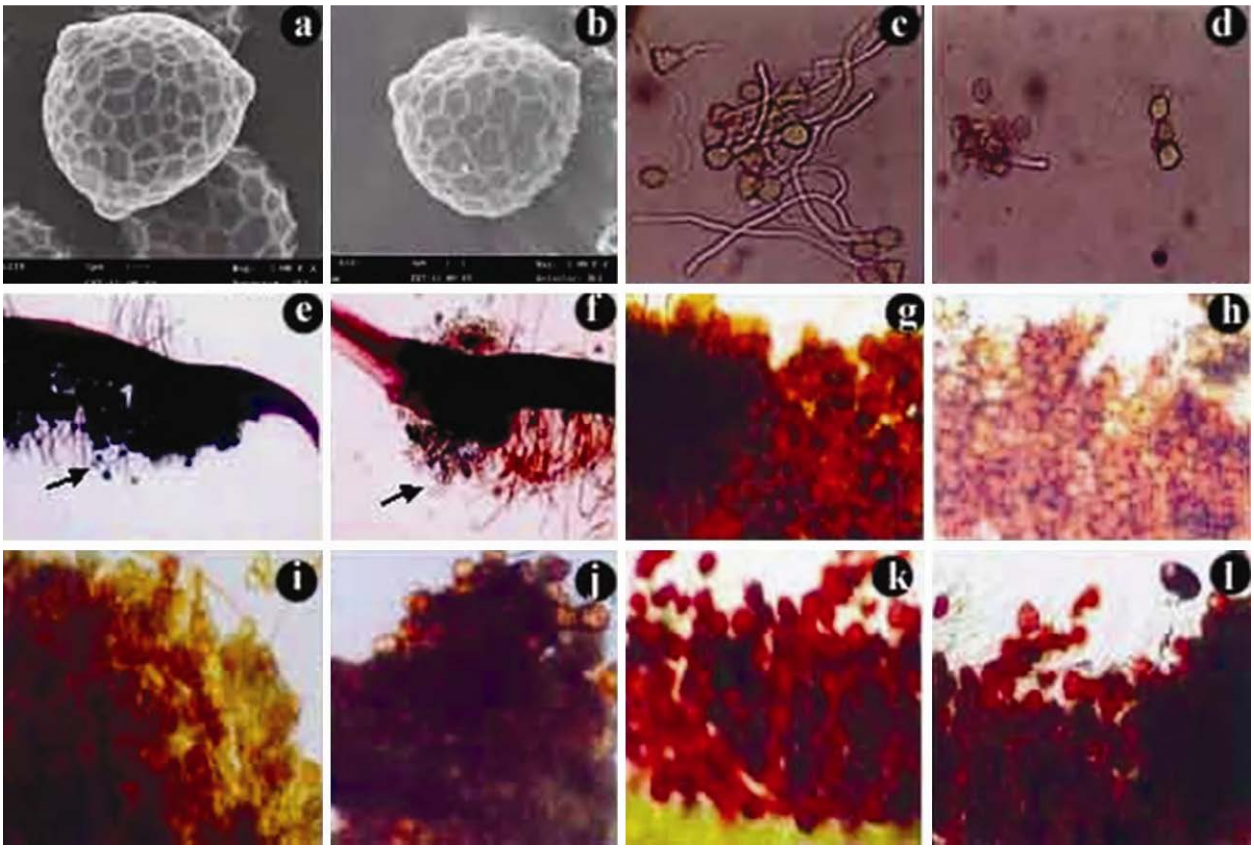

Fig. 1— SEM of pollen grains of control (a) and Zn deficient (b) black gram plants. *In vitro* germination of control (c) and Zn deficient (d) pollen grains. Germinating pollen grains (arrow) on the stigma of control (e) and Zn deficient (f) black gram plants. Histochemical localization of esterase (g, h), peroxidase (i, j) and acid phosphatase (k, l) on stigma of control (g, i, k) and Zn deficient (h, j, l) black gram plants.

hydration and pollen tube growth in plants[22]. These changes were specific to Zn deficient plants and were also observed in lentil[5] and black gram[6]. Less number of pollen grains adhering to stigma reduced the pollen tubes competition to reach ovule and finally seed set which was ameliorated by foliar application of Zn especially when given at initiation of bud formation (Zn+F2). The decrease in esterase activity in Zn deficient stigma is directly related with a lack of stigmatic exudates followed by limiting pollen germination, pollen-stigma interaction and fertilization[23]. Acid phosphatase and peroxidase are hydrolytic enzymes and their activity increased under Zn deficiency, which inhibited pollen tube growth[24] leading to poor fertilization and seed set, which was overcome by foliar Zn supply. Foliar application of Zn has been reported to be a short term effective approach not only for improving yield in deficient soils but also to achieve nutritional efficacy and has been carried out in cereals[10, 25, 26].

Seeds harvested from Zn deficient plants showed poor carbohydrates content. Low sugars in Zn deficient condition is directly related to reduced photosynthetic and aldolase activity which limits the conversion of fructose 1-6-diphosphate to its subsequent compounds and thus creates a poor correlation between source and sink of plant[27]. The activity of sucrose synthase, an enzyme required for sugar synthesis which plays an important role in seed filling and seed size[28] was also reported to be decreased under Zn deficiency. The decrease in starch concentration in seed of Zn deficient plants, as a consequence of which deformed and under developed seed were produced could be due to retarded activity of starch synthase[29]. Improved starch content in seeds by additional Zn would be due to increased activity of starch synthase and starch synthesis as a consequence of which viable and fully developed seed were produced after foliar application of Zn resulting in increase in seed weight and viability.

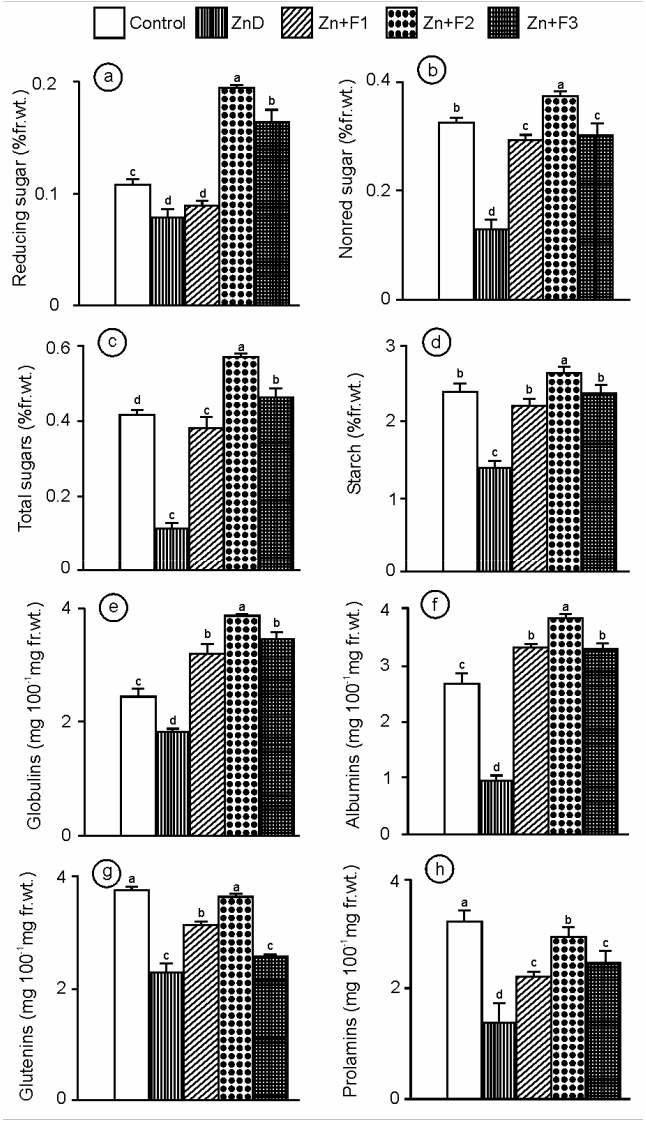

Fig. 2 —Effect of foliar Zn supply on the concentration of reducing (a), non-reducing (b), total sugars (c), starch (d) and seed protein fractions – globulins (e), albumins (f), glutenins (g) and prolamins (h) in black gram plants. Bars indicate mean ± SE (n=3). Different letters denote significance at LSD $P \leq 0.05$.

The decrease in storage protein concentration in seed of Zn deficient plants might be due to a sharp increase in RNase activity in Zn deficient plants which ultimately results in increased accumulation of amino acid. This was overcome by foliar spray of Zn which is known to keep enzyme active by binding the sulphydryl group and thus protecting disulphide formation which leads to increase in protein synthesis[30]. The albumins and globulins, which are more abundant in legumes than the prolamins and glutenins[31] responded better to foliar Zn and increased to values more than in the control plants.

The seed Zn content was also significantly increased by foliar Zn supply to concentrations more than the control values. Our results indicate that high seed Zn is important to carry out physiological roles during seed germination and early seedling growth and thus seeds of Zn+F1, Zn+F2 and Zn+F3 plants showed enhanced seed viability. This is in agreement with reports[32] that seeds from plants grown with adequate Zn supply show enhanced seedling growth and serve as a starter fertilizer in Zn deficient soils. The seeds of black gram plants fortified with foliar Zn have high seed Zn density and protein content which would be highly beneficial for human consumption. Zinc enriched seeds are important not only for human consumption but they also improve crop yield in Zn deficient soil, by providing better seedling growth, abiotic stress tolerance and pathogen resistance.

In conclusion, we can say that Zn has a significant effect on plant reproductive structure development, seed yield and seed quality. Foliar application of Zn is an effective tool for biofortification as it significantly improved seed yield and quality of black gram. In the present study the time of foliar application was also found to be extremely important. The foliar application of 0.1% Zn SO_4 prior to bud initiation was found to be most beneficial for enhancement of floral characters, seed yield and quality of seeds in terms of Zn density, carbohydrate and protein content.

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