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Seed Priming and Foliar Spray with Nano Zinc Improves Stress Adaptability and Seed Zinc Content without Compromising Seed Yield in Ragi (Finger millet)

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ABSTRACT

The decrease in nutritional content in the edible portion of crop plants can exuberate existing wide spread nutritional deficiency disorders. In particular, zinc (Zn) is being one of the important micronutrient for plant and human nutrition, deficiency is considered to be one of the leading risk factor. To address these problems, we have to think of a new technology like Nanotechnology to precisely detect and deliver the correct quantity of nutrients or other inputs required by crops that promote productivity and environmental safety. Nano-fertilizers are nutrient carriers of nano-fertilizers having <100 nm in size and they are capable of holding higher nutrient ions due to their high surface area. Nano-fertilizers and nanocomposites can be used to control the release of nutrients from the fertilizer granules so as to improve the nutrient use efficiency while preventing the nutrient ions either get fixed or lost to the environment. With this background we studied the effect of Zinc oxide (ZnO) nano particle on finger millet which is one of the important millet crops widely grown in southern India. Initially we standardized the Zn concentration for the seed priming with different concentration. 1000ppm of ZnO nano showed higher germination percent, root and shoot length compared to common $ZnSO_4$ and Zn Gluconate. Further crop was grown in the pot culture experiment with different treatment and different method of application of Zn. ZnO nano treated plant with combination of seed priming and foliar application showed higher plant height, number of tillers, chlorophyll content and increased yield with high leaf and seed zinc content.

Key words: *Nano-fertilizers, Nutrients, Micronutrient, Chlorophyll.*

INTRODUCTION

Exponential growth of the population, the expectations for higher grain productivity at present and in the future may cause decreased mineral contents in the grains[1,2]. A challenge for global food and nutrition security is to feed the world population with nourishing food[3,4]. Therefore in the future, emphasis laid on production of high quality food with the required level of nutrients and proteins[5,6].

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Micronutrient deficiencies in humans as well as crop plants are difficult to diagnose and consequently the problem is termed as 'hidden hunger'[7]. This hidden hunger may cause nearly 40% reduction in crop productivity and also estimated that it affects more than a half of the global population. Micronutrient deficiencies in general refers to iron (Fe) Zinc (Zn), Selenium (Se), Iodine (I), Cupper (Cu), and Mg[1], among them Zn deficiency is most wide spread nutritional disorder next to Fe, Vitamin A and Iodine. World Health Organisation (WHO) reported that the Zn deficiency stands fifth risk factor for causing diseases among children's in developing countries. Based on analysis of diet composition and nutritional needs, it has been estimated that 49% of the world's population (equivalent to 3 billion) are at risk of suffering from Zn deficiency.

Zn has prominent physiological functions in all living systems, such as maintenance of structural and functional integrity of biological membranes, as a cofactor for more than 300 enzymes, detoxification of highly toxic oxygen free radicals and contribution to protein synthesis and gene expression under normal and stress conditions etc. Zn is needed by the largest number of proteins for attain structural property, at least 2800 proteins are Zn dependent and make up nearly 10% proteomes in eukaryotes. Zn has a vital role in several body functions such as vision, taste perception, cognition, cell reproduction, growth and immunity, resistance to some infectious diseases such as diarrhoea[8] and immunity[9].

In order to overcome Zn disorder, several strategies are being employed such as supplementation, fortification, diversification and biofortification. Among these strategies biofortification of food crops with Zn is considered to be cheaper and sustainable. The simplest of these techniques to increase Zn content of plants is through the addition of the appropriate mineral as an inorganic compound to the fertilizer. This method has been successful in many instances but depends on the crop species, cultivar, the mineral itself, quality and properties of the soil, making the strategy difficult to apply generally. The major advantages of this method is simple, inexpensive and enhancement can be achieved very rapidly.

However Zn being a heavy metal, indiscriminate application of Zn fertilizers to soil over years will lead to accumulation in soil to the levels toxic to the plants. With the current heavy emphasis on Zn in agriculture, care should be taken not to get over zealous with Zn applications. Therefore, an efficient mechanism to reduce the amount of Zn fertilizer application to soil/ canopy without compromising the plant growth and yield is very essential. Hence, in recent years the application of nano scale particle of Zn is being preferred to enhance the uptake by plants by effecting agronomic effectiveness of Zn fertilizers.

Nanotechnology has been described as the next great frontier of agricultural sciences[10] and occupies a prominent position in transforming agriculture and food production through efficient management of soil nutrients[11]. Because nano particles are spherical or faceted metal particles typically < 100nm in size. These nano particles are having high surface area (30-50 m^2/g), high activity, better catalytic surface, rapid chemical reaction, rapidly dispersible and adsorb abundant water. So nano fertilizers may increase the efficiency of nutrient uptake, hence enhance yield and nutrient content in the edible parts and also minimize its accumulation in the soil. The development of nano materials could open up the novel applications in plant biotechnology and soil science. It is anticipated that very soon the industrial production of manufactured nano particles will be increased by manifold and released into the market. However with significant potential benefits, there are considerable uncertainties with regards to potential risks to the environment and human health that needs to be clarified. In the present study an attempt has been made to study the effect of Zn oxide (ZnO) nanoparticles on ragi crop growth, yield performances and crucially seed Zn content.

MATERIALS AND METHODS

ZnO nano suspension preparation and standardization of Zn concentration for seed priming

ZnO nanoparticles of 30nm size procured from SRL Pvt Ltd Company from Maharastra was used in this study in comparison with $ZnSO_4$ and Zn gluconate as a reference Zn source. Zinc gluconate is the zinc salt of gluconic acid. It is an ionic compound consisting of two anions of gluconate for each zinc (II) cation. ZnO nanoparticles were dissolved by suspending directly in deionized milli Q water and dispersed by ultrasonic vibration (100 W, 40 KHz) for 30 min. The nano scale suspensions appears as a clear solutions at pH of 6.8-7 were used. Magnetic bars were placed in the suspensions for stirring before use to avoid aggregation of the particles.

For concentration standardizing, ZnO nano suspension, Zn gluconate and $ZnSO_4$ solutions of 500, 1000 and 1500 ppm concentrations were prepared. The ragi seed (GPU 28) were soaked in different concentrations of Zn solutions for five hours and were shade dried for 1 hour. The seeds were then placed in petri plate (100x15mm) with a single layer of sterilized filter paper added with 5 ml of water (as per the recommendations of the International Seed Testing Association, 1976). Petri plates were covered and placed in an incubator at 26 ± 1˚C. After seven days of incubation, the germination percentage, root length and shoot length were recorded. Seedling Vigor Index (SVI) was calculated using the formula[12].

SVI= (Germination percent x (Root length + Shoot length))

Zinc treatment imposition in pot experiment and water stress treatment

The plants raised in the pots filled with the equal proportions of standard pot filling mixtures of soil, sand and FYM were maintained under the rain out shelter. The treatments include seed priming (500ppm), foliar application (ZnO nanoparticle 2g/15L, and Zn gluconate, $ZnSO_4$ 30g/15L) and combination of seed priming + foliar application. The foliar application was taken up at 30 and 60 days after sowing. Another set of plants were maintained with same treatments and water stress was imposed at 45 days after sowing (DAS) for 5 days till soil moisture reached to 70 percent field capacity. Growth and yield observations like plant height, number of tillers, SPAD chlorophyll, percent membrane leakage, relative water content (RWC), yield per plant, test weight and total plant dry matter were recorded.

Estimation of Zn content

Zn content in seed and leaf samples were analysed using inductively coupled plasma optical emission spectrometer (ICP-OES). The samples were collected from different treatments were oven dried. Sample preparation and analysis was done using the protocol followed in[13].

Statistical analysis

Experimental data were analysed using MSTAT-C statistical programme at 5 % significance level of in a RCBD three factorial design. The analysis of variance (ANOVA) test was used to demonstrate the effect of Zn treatments on the growth, yield parameter and Zn content of ragi.

RESULT AND DISCUSSION

Standardization of Zn concentrations for seed priming

Out of three concentrations used for seed priming, 500 ppm of ZnO, Zn gluconate and $ZnSO_4$ was found to be optimum in promoting seed germination. Significant increase in germination percentage, root length and shoot length was observed in all the Zn sources. SVI of ragi treated with different concentration of Zn were showed in (Fig 1). ZnO nano was found to be more effective in enhancing the seed germination and seedling vigor compared to other Zn sources at 500ppm concentration. Similarly groundnut seeds primed using ZnO nano particles prior to incubation for germination showed significant increased germination percentage and SVI[14]. Seedling vigor of germinating seed chiefly depends on the seed stored nutrients and several reports showed that the plants emerging from the seeds with low Zn have poor seedling vigor[15]. High seed Zn content in wheat has significantly improved the root and shoot growth under Zn deficiency affected soil[16]. Seed priming with Zn for barley seeds showed more effective in improving seed germination and seedling development[17]. Therefore the seed priming with ZnO nano particle can enhance the germination percent and seedling vigor index hence better establishment of crop growth at the initial growth stages.

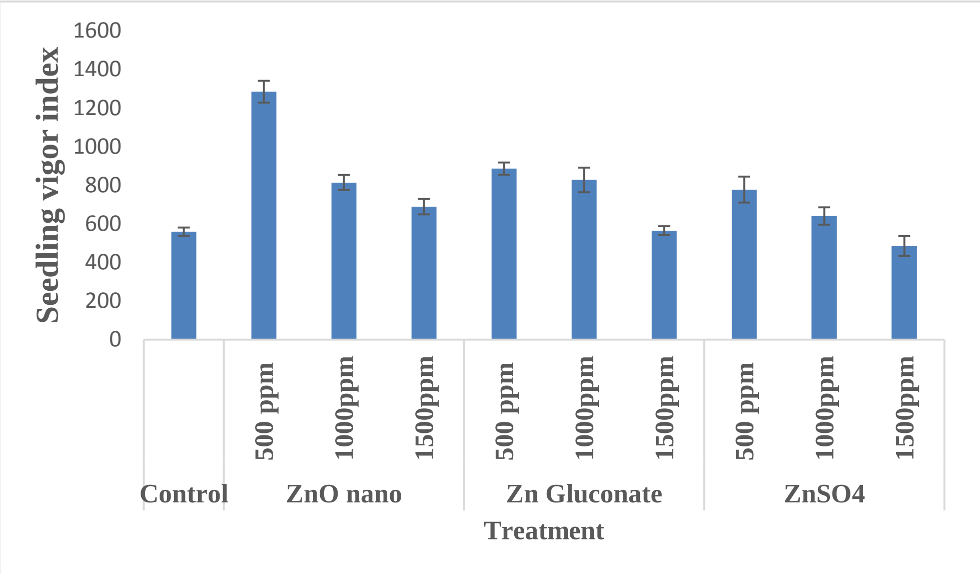

Fig. 1: Seedling vigor index (SVI) of ragi treated with ZnO nanoparticles (ZnO), Zn gluconate and $ZnSO_4$ at different concentrations

Physiological, growth and yield observations of ragi plants under well watered and water stress conditions

The ragi plants maintained in pot with Zn treatment showed better physiological growth and yield performances compared to control plants under well watered conditions. Although the physiological parameters like chlorophyll (SPAD), RWC were improved and decrease in the membrane leakage in ZnO nano treated was noted than Zn Gluconate and $ZnSO_4$. Similarly the growth and yield observations like plant height, number of leaves, yield per plant, test weight, shoot biomass were increased significantly in ZnO nano treatment compared to Zn gluconate and $ZnSO_4$ (Table.1). Out of three method of application used for Zn treatment combination of seed priming with foliar application was found to be more effective than seed priming or foliar application alone in all the Zn sources. Since the initial seed priming treatment will improve the seedling vigor and helps in better establishment of the plant[18] and foliar application during vegetative and reproductive stage will supply the Zn to the developing seed and hence can improve the growth and also Zn content in the plant[19]. In Groundnut, application of Zn through foliar application was found to be more effective compared to soil application in improving the growth performances[14]. An increase in yield of about 21.4 % was observed in ZnO nano treated plants, over $ZnSO_4$ in combination of seed priming and foliar application treatment.

Table 1: Performance of Ragi, as influenced by moisture levels, different sources of zinc and method of application

Treatments	Plant height(cm)	Number of Tillers /plant	Chlorophyll (SPAD)	Membrane leakage (%)	RWC (%)	Ear head wt (gm) /plant	Yield / plant (g)	Test weight (gm)/ (1000 seeds)	Shoot biomass (gm) / plant
Water Stress									
Control	95.8	3.72	30.9	58.3	80.7	23.6	19.9	3.09	53.6
Stress	89	3.61	30.3	56.8	79.9	21.8	18.1	2.87	49.6
SEM ±	**0.34**	**0.07**	**0.343**	**0.207**	**0.27**	**0.09**	**0.058**	**0.079**	**0.5**
CD (P=0.05)	**2.03**	**NS**	**NS**	**1.26**	**NS**	**0.54**	**0.353**	**NS**	**1.42**
Zinc sources									
Control	85.3	3.55	29.3	60.9	77.7	17.5	15.1	2.73	41.6
ZnO nano	105	3.77	31.3	54.6	82.4	29.3	21.5	3.21	63
Zn gluconate	95.8	3.66	30.5	55.2	80.8	24.3	18.7	2.66	54.9
$ZnSO_4$	85.9	3.66	31.2	59.5	80.3	19.7	17.7	3.32	46.9
SEM ±	**0.48**	**0.137**	**0.252**	**0.234**	**0.205**	**0.22**	**0.181**	**0.051**	**0.49**
CD (P=0.05)	**1.37**	**NS**	**0.718**	**0.667**	**0.584**	**0.64**	**0.516**	**0.145**	**1.39**
Method of applications									
Seed treatment	89	3.75	31.1	57.6	81.9	22.8	19.03	2.97	51.7
Seed priming + foliar spray	94.6	3.66	30.5	59.5	78.5	22.5	18.7	3.02	51.3
Foliar spray	93.7	3.58	30.1	55.6	80.8	22.7	19.4	2.95	51.9
SEM ±	**0.42**	**0.119**	**0.219**	**0.202**	**0.177**	**0.19**	**0.516**	**0.044**	**0.42**
CD (P=0.05)	**1.18**	**0.339**	**0.624**	**0.576**	**0.504**	**NS**	**NS**	**NS**	**NS**
CV (%)	**2.21**	**15.95**	**3.51**	**1.73**	**1.08**	**4.19**	**4.04**	**7.28**	**4.02**

The results indicated the higher efficiency of Zn absorption was observed in ZnO nano particle treatments due to the surface properties of the nano particles and hence the tissue showed higher Zn content. Similar results with the use of ZnO nano in field grown groundnut was reported[14]. But all the Zn treated plants showed a significant affirmative response compared to the untreated control plants which explains the physiological importance of optimum concentrations of Zn in maintaining plant metabolism.

Under water stress treatment at vegetative stage, the ragi plants showed reduced growth and yield compared to control plants. Even though stress was imposed for the shorter period but found more critical in affecting the plant growth and yield performances. Importance of Zn nutrition on water relations of crop plant is likely to be related to the severity of Zn deficiency and water deficit conditions. In chickpea the decreased root growth during Zn deficiency has reduced the plants ability to exploit soil moisture reserves[20]. However with the Zn treatment either with ZnO nano, Zn gluconate and $ZnSO_4$, to stresse imposed plants showed improved phenotype although ZnO nano particles found more effective in reducing the stress effect on ragi plant. Improved chlorophyll, relative water content and less percent membrane leakage was observed as an indicator of stress tolerance in the Zn treatment imposed plants (Table 1). Similarly upon Zn supplementation to the field grown chickpea when the soil moisture reserves were declining, an increase in water potential and stomatal conductance was observed[21]. During water stress ZnO nano supplementation with any method of application to the water stress imposed ragi plant found more effective in improving growth and yield performance than seed priming or foliar application alone. With the Zn treatment irrespective of control or stress condition the seed Zn content in ragi plants was significantly high compared to untreated plants. In our study we found that ZnO nano treatment with seed priming and foliar application in both control and stress condition have significant increase in the yield as compared to other zinc sources (Table 2).

Analysis of Zn content

The ragi plants supplemented with ZnO nano particle Zn Gluconate and $ZnSO_4$ were analyzed for leaf Zn and seed Zn content by using ICP-OES. The results showed the improved leaf and seed Zn content in all the Zn treatment compared to control plants. Under well watered condition highest seed and leaf Zn content of 13.9 mg/100g and 8.94 mg/100g was found in ZnO nano treatment in seed priming and foliar treatment combinations. Similarly under water stress condition also the improved leaf Zn content of 12.55mg/100g and seed Zn content of 7.4mg/100g was observed in seed priming with foliar application treatment (Fig 2). ZnO nano treated plants showed an increase in leaf Zn content of 39.71% and seed Zn content of 62.63% over $ZnSO_4$. The result indicated the better transport and accumulation Zn in seed and leaf with ZnO nano treatment compared to $ZnSO_4$ treatment.

Table 2: Interaction effect of water stress treatment, different zinc sources and method of application on grain yield per plant of ragi (g/plant).

Water Stress treatment + different zinc sources	Method of application		
	Seed priming	Seed priming + foliar application	Foliar application
Control + 0	14.2	14.3	14.5
Control + ZnO nano	26.5	27.5	24.7
Control + Zn gluconate	24.3	19.7	17.5
Control + $ZnSO_4$	16.7	18.3	20.1
Stress + 0	11.9	11.6	12.8
Stress + Zno nano	23.8	24.9	25.9
Stress + Zn gluconate	20.3	19.2	23.0
Stress + $ZnSO_4$	14.5	13.4	17.3
SEM±	**0.443**		
CD(P=0.05)	**1.26**		
CV (%)	**4.04**		

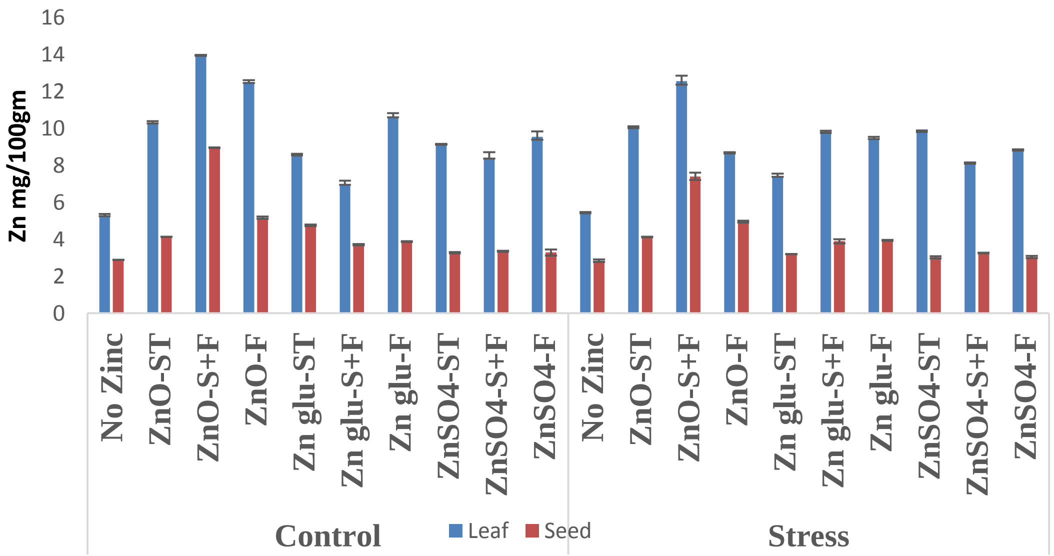

Fig 2: Leaf and seed zinc content of ragi crop maintained in control and stress condition with different zinc sources and treatment like seed priming (ST), seed priming and foliar application (S+F) and foliar application (F)

CONCLUSION

Delivering the micronutrient to ragi plant through nano size has significant effect on improving growth, yield performance and seed Zn content. Nutrient requirement for crop growth is developmentally regulated therefore proper application of Zn during different growth stages is crucial. In the present study the combinations of seed priming before sowing and foliar application at later growth stages with Zn proved to be more effective in increasing yield and seed Zn content. Overall the study highlighted the importance of ZnO nano particle in improving the growth and yield performance together with increasing the Zn content in the edible parts of ragi plant. This is one of the promising strategy to mitigate the Zn malnutrition through increasing the Zn content in the food grains although there is a scope to understand the penetration and transport mechanism of ZnO nano inside the plant system.

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