BIOFORTIFICATION OF WHEAT USING BIOLOGICALLY SYNTHESIZED ZINC NANOPARTICLES

ABSTRACT

The experiment was conducted at the Nanotechnology Laboratory, University of Agricultural Sciences, Dharwad, Karnataka. The zinc nanoparticles were biosynthesized through *Pseudomonas* and actinobacteria and the biosynthesized nanoparticles were characterized using UV-Visible spectroscopy, Particle Size Analyzer (PSA), Scanning Electron Microscope (SEM), (EDX), X-Ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). After biosynthesis of the nanoparticles (NPs), a greenhouse experiment was conducted under controlled condition to enrich the zinc content in wheat using biosynthesized zinc nanoparticles. In wheat seed priming at 500 ppm and foliar spraying at 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through actinobacteria (T_{12}) recorded significantly higher grain yield (3.95 g plant^{-1}), straw yield (5.97 g plant^{-1}), zinc content (grain 55.87 µg g^{-1} and straw 66.27 µg g^{-1}) and zinc uptake (grain 220.69 µg plant^{-1}, straw 395.63 µg plant^{-1} and total zinc uptake 616.32 µg plant^{-1}), nitrogen uptake (115.98 mg plant^{-1}), phosphorus uptake (28.34 mg plant^{-1}) and potassium uptake per plant (97.55 mg plant^{-1}) which was on par with seed priming at 500 ppm and foliar spraying at 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through *Pseudomonas* (T_5).

Key Words: Biosynthesis, foliar spraying, Pseudomonas, wheat

Introduction

Zn is essential for the synthesis and activation of several hormones (auxin and gibberellin) and enzymes that enhance seed germination per cent and seedling growth. Additionally, Zn plays a important role in biosynthesis of proteins, carbohydrates, lipids, and nucleic acids in plants (Sturikova *et al.,* 2018). Zn can serve as a cofactor for P-solubilizing enzymes like phosphatase and phytase, and nano-ZnO boosted their activity in the soil (Raliya *et al.,* 2015). Unlike chemical fertilisers, nanofertilizers can be synthesized and applied based on the crop's nutritional needs and the status of the soil's nutrient levels using biosensors (Kah *et al.,* 2018). Nanofertilizers using nanomaterials because of their high surface-to-volume ratio, gradual and controlled release at target places (Feregrino-Perez *et al.,* 2018). Additionally, nanofertilizers, as opposed to chemical fertilisers, allow for high mineral bioavailability to plants due to their smaller size, greater reactivity, and higher surface area (Liu and Lal, 2015).

The most of the wheat types grown today are nutrient poor, especially in Fe and Zn. The most of these minerals are lost during the milling process, which makes them scarce in the human diet and causes malnutrition. Nearly two billion people worldwide, especially those in Asia and Africa, suffering from hidden hunger due to their reliance on cereal crops, particularly wheat, for their daily diet (Prom-U-Thai *et al.,* 2020). These deficits are particularly widespread among the growing children, pregnant and lactating women and manual labours in highly developed countries. Zn and Fe are the micronutrients that are most commonly linked to micronutrient deficiency globally. Low availability of microelements in soil may be a contributing factor to the decreased level of essential nutrients in crops, due to sub optimal abiotic circumstances, such as abnormally high or low temperature, pH, a lack of water, anaerobic conditions, and the presence of other components (Manojlovic *et al.,* 2019). The prevalence of micronutrient deficiencies is higher in humid temperate and tropical regions, where there is extensive leaching caused by excessive precipitation. Another factor is the usage of plant types which have a poor capacity to store adequate amounts of microelements in their edible parts (Jalal *et al.,* 2020).

Increased micronutrient levels in crops can be achieved through biofortification (Dhaliwal *et al.,* 2022). Biofortified crops have been demonstrated to increase the consumption of micronutrients and significantly improve human health (Praharaj *et al.,* 2021). The three main strategies for biofortification are agronomic, conventional plant breeding, and plant breeding utilising genetic engineering. Agronomic biofortification is considered to be the simplest way to increase the levels of microelements in crops because it focuses on supplying micronutrients that can be directly absorbed by the plant through application with mineral or foliar fertilisers or the improvement of the solubilization and mobilisation of mineral elements in the soil. One of the most affordable methods to lessen dietary mineral deficit in humans is agronomic biofortification (Szerement *et al.,* 2022).

MATERIAL AND METHODS

Zinc nanoparticles were biosynthesised at the University of Agricultural Sciences, Dharwad, in the Nanotechnology Laboratory. Actinobacterial and pseudomonas isolates were gathered and examined at the Microbial Genetics Laboratory, Department of Agricultural Microbiology, UAS, Dharwad. In the Institute of Agri-Biotechnology (IABT) polyhouse at the University of Agricultural Sciences, Dharwad, the pot experiment was carried out in 2022-2023. A soil sample was collected in the polybags. The soil was ground into a powder, dried, and put through a 2 mm sieve before its chemical and physical analysis. The experimental soil's textural class was clayey, with a pH of 7.76, an EC of 0.27 dS m^{-1}, organic carbon at 0.49 %, accessible nitrogen at 251.37 kg ha^{-1}, phosphorus at 31.45 kg ha^{-1}, potassium at 363.24 kg ha^{-1}, zinc at 0.53 ppm, and iron at 6.78 ppm. In the Main Agricultural Research Station, Dharwad collected the UAS 334 variety of wheat. Five seeds were planted in each pot. For the appropriate treatments, seeds were primed for six hours with a 500 ppm solution of biosynthesised zinc nanoparticles. The application of urea, diammonium phosphate, and muriate of potash, respectively, provided nitrogen, phosphorus, and potassium. The experiment was conducted using a Completely Randomized Design (CRD) with sixteen treatments replicated three times. Details of the experiment were T_1- seed priming with BS (Bacterial (*Pseudomonas*) synthesized) ZnNPs at 500 ppm; T_2- foliar spraying with BS ZnNPs at 500 ppm; T_3- foliar spraying with BS ZnNPs at 1000 ppm; T_4- foliar spraying with BS ZnNPs at 1500 ppm; T_5- seed priming at 500 ppm + foliar spraying at 500 ppm with BS ZnNPs; T_6- seed priming at 500 ppm + foliar spraying at 1000 ppm with

BS ZnNPs ; T_7- seed priming at 500 ppm + foliar spraying at 1500 ppm with BS ZnNPs; T_8- seed priming with ABS (Actinobacteria synthesized) ZnNPs at 500 ppm; T_9- foliar spraying with ABS ZnNPs at 500 ppm; T_{10}- foliar spraying with ABS ZnNPs @ 1000 ppm; T_{11}- foliar spraying with ABS ZnNPs at 1500 ppm; T_{12}- seed priming at 500 ppm + foliar spraying at 500 ppm with ABS ZnNPs; T_{13}- seed priming at 500 ppm + foliar spraying at 1000 ppm with ABS ZnNPs ; T_{14}- seed priming at 500 ppm + foliar spraying at 1500 ppm with ABS ZnNPs; T_{15}- RDF (100:75:50, $N:P_2O_5:K_2O$ kg ha^{-1}, respectively) and T_{16}-control (No fertilizer application). For all foliar treatments, foliar spraying is done at panicle initiation stage. RDF common for all treatments, with the exception of control.

Grain yield and straw yield

The total yield from each plant was recorded and was presented as grain yield per plant. Straw yield per plant was worked out for respective treatment and was presented as straw yield per plant. The data on grain yield and straw yield were used to calculate the harvest index by using the formula given by Donald (1962).

Methodology used for plant analysis

Preparation of sample	Destructive plant sample for dry matter estimation was used for plant analysis. Nitrogen, phosphorus, potassium, zinc and iron content in whole plant were analyzed at the end.
Nitrogen	Nitrogen uptake on dry weight basis was determined by modified Kjeldahl's method as described by Jackson (1973).
Phosphorus	Plant samples were digested with triacid mixture. The phosphorus uptake on dry weight basis was determined by Vanado-molybdic phosphoric acid yellow colour method in HNO_3 (Jackson, 1967).
Potassium	Potassium uptake on dry weight basis was determined by feeding digested plant sample to flame photometer (Jackson, 1973).
Zn and Fe	AAS method (Lindsay and Norvell, 1978).

Nutrient content and uptake

The wheat grains were crushed with a grinder. Initially, the samples were digested using HNO_3 and $HClO_4$.After digestion with HNO_3 and $HClO_4$, the samples were analyzed for nutrient concentrations using an atomic absorption spectrometer as given by Lindsay and Norvell in 1978. The nutrient uptake was calculated based on the plants' nutrient content and dry matter production, using the following formula.

$$\text{Nutrient uptake (g plant}^{-1}) = \frac{\text{Nutrient concentration (\%)}}{100} \times \text{dry matter (g plant}^{-1})$$

Statistical analysis

The data collected from the experiment at various growth stages were subjected statistical analysis following the method given by Gomez and Gomez (1984). The significance level used in the 'F' test was P = 0.01 (1%). The critical difference (CD) at 1% levels was computed whenever the 'F' test was given significant results. The mean values of treatments were separately subjected to Duncan Multiple Range Test (DMRT) using the corresponding error mean sum of squares and degrees of freedom.

RESULTS AND DISCUSSION

Effect of biosynthesized zinc nanoparticles on wheat yield

Seed priming @ 500 ppm and foliar spraying @ 500 ppm at panicle initiation stage with ZnNPs biosynthesized from actinobacteria and *Pseudomonas* increased the wheat yield per plant by 44.16 and 41.97 per cent, respectively than control (Table 1). A sufficient Zn supply boosted the absorption of N at the stage of grain formation, which eventually improved yield (Dhaliwal *et al.*, 2022). Zinc promotes the synthesis of auxin, which improves the absorption of nitrogen and eventually results in enhanced yield and yield characteristics. The synthesis of carbonic an hydrase enzyme is increased by Zn treatment, which maximises nutrient intake and grain yield by increasing photosynthetic activity in leaves. Zn involvement in the biosynthesis of indole acetic acid, particularly in the initiation of primordial reproductive parts and the allocation of photosynthates towards them, might have an impact on the improved yield and yield components (Jangid *et al.*, 2019). Adhikari *et al.* (2016) revealed that application of ZnONPs raised the chlorophyll content, and the increased chlorophyll content had a beneficial impact on net photosynthesis as well as increasing dry weight and improving yield.

Effect of biosynthesized ZnNPs on nutrient content and nutrient uptake in wheat

Wheat seed priming at 500 ppm and foliar spraying at 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through actinobacteria (T_{12}) recorded significantly highest zinc content (grain 55.87 µg g^{-1} and straw 66.27 µg g^{-1}) and zinc uptake (grain 220.69 µg $plant^{-1}$, straw 395.63 µg $plant^{-1}$ and total zinc uptake 616.32 µg $plant^{-1}$) and nitrogen uptake (115.98 mg $plant^{-1}$), phosphorus uptake (28.34 mg $plant^{-1}$) and also potassium uptake per plant (97.55 mg $plant^{-1}$) which was on par with seed priming at 500 ppm and foliar spraying at 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through *Pseudomonas* (T_5) (Table 2, 3 & 4). Zn foliar spraying near the heading stage resulted in increasing the absorption and translocation towards the grain for grain development. Foliar application of Zn nanoparticles was most effective at enhancing physiological parameters like chlorophyll content, total soluble sugar, carbonic anhydrase, and grain phytase activity that resulted in the highest grain Zn concentration (Moshfeghi *et al.,* 2020). As compared to ordinary zinc sulphate, foliar spraying with ZnNPs particles might be more effective due to their larger surface area, which led to increased activity, ion adsorption and faster chemical reaction (Tarafdar *et al.* 2014). Concentration of Zn in the wheat grain recorded higher with foliar application of nano zinc due to increased translocation of Zn in grains as compared to traditional zinc sulphate (Dapkekar *et al.,* 2018 and Doolette *et al.,* 2020). Because of their smaller size and greater surface area, which allowed for increase in the zinc absorption and finally it increases the zinc concentration in grain (Raddy *et al.,* 2018.). The higher Zn content in grains was noted with foliar sprayed zinc nanoparticles as compared to conventional foliar treatments. This may be due to greater uptake and absorption from leaf surfaces as well as greater translocation from the place of application to other plant portions which are deficient in zinc (Umar *et al.,* 2021). Poornima and Koti (2019) reported that compared to bulk zinc, seed primed with nano zinc increased the grain zinc concentration, might be due to nano scale zinc oxide has a higher uptake and translocation efficiency than bulk zinc form, also reported that zinc nanoparticles with high specific surface and surface reactivity not only easily adsorbed on physical surfaces but also reacted with biological proteins and resulted in their uptake and subsequent quick and efficient translocation to the sink.

Table 1. Grain yield, straw yield and harvest index of wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	**Yield**		
	Grain yield (g plant^{-1})	**Straw yield (g plant^{-1})**	**Harvest index**
T_1- SP with BS ZnNPs @ 500 ppm	3.21^b	5.13^b	0.38^a
T_2- FS with BS ZnNPs @ 500 ppm	3.34^b	5.32^b	0.39^a
T_3- FS with BS ZnNPs @ 1000 ppm	3.28^b	5.24^b	0.38^a
T_4- FS with BS ZnNPs @ 1500 ppm	2.75^c	4.62^c	0.37^a
T_5- SP + FS with BS ZnNPs @ 500 ppm	3.89^a	5.93^a	0.40^a
T_6- SP + FS with BS ZnNPs @ 1000 ppm	3.80^a	5.81^a	0.40^a
T_7- SP + FS with BS ZnNPs @ 1500 ppm	2.80^c	4.67^c	0.37^a
T_8- SP with ABS ZnNPs @ 500 ppm	3.26^b	5.17^b	0.39^a
T_9- FS with ABS ZnNPs @ 500 ppm	3.40^b	5.38^b	0.39^a
T_{10}- FS with ABS ZnNPs @ 1000 ppm	3.35^b	5.26^b	0.39^a
T_{11}- FS with ABS ZnNPs @ 1500 ppm	2.78^c	4.64^c	0.37^a
T_{12}- SP + FS with ABS ZnNPs @ 500 ppm	3.95^a	5.97^a	0.40^a
T_{13}- SP + FS with ABS ZnNPs @ 1000 ppm	3.87^a	5.85^a	0.40^a
T_{14}- SP + FS with ABS ZnNPs @ 1500 ppm	2.82^c	4.70^c	0.38^a
T_{15}- RDF (100:75:50, N: P_2O_5: K_2O kg ha^{-1})	2.74^c	4.61^c	0.37^a
T_{16}- Control	1.25^d	2.28^d	0.35^a
S.Em.±	**0.08**	**0.10**	**0.008**

SP-Seed priming; **FS**-Foliar spraying; **BS**-Bacterial (*Pseudomonas*) synthesized; **ABS**-

actinobacterial synthesized; Seed priming @ 500 ppm common for all seed primed treatments; RDF common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.01)

Table 2. Zinc concentration in wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	**Zinc concentration ($\mu g\ g^{-1}$)**	
	Grain	**Straw**
T_1- SP with BS ZnNPs @ 500 ppm	47.59^c	62.13^b
T_2- FS with BS ZnNPs @ 500 ppm	51.62^b	61.46^b
T_3- FS with BS ZnNPs @ 1000 ppm	50.57^b	60.57^b
T_4- FS with BS ZnNPs @ 1500 ppm	43.35^d	56.78^c
T_5- SP + FS with BS ZnNPs @ 500 ppm	54.96^a	65.26^a
T_6- SP + FS with BS ZnNPs @ 1000 ppm	54.83^a	64.73^a
T_7- SP + FS with BS ZnNPs @ 1500 ppm	44.92^d	57.62^c
T_8- SP with ABS ZnNPs @ 500 ppm	48.57^c	62.35^b
T_9- FS with ABS ZnNPs @ 500 ppm	52.21^b	61.87^b
T_{10}- FS with ABS ZnNPs @ 1000 ppm	51.73^b	60.95^b
T_{11}- FS with ABS ZnNPs @ 1500 ppm	43.69^d	57.34^c
T_{12}- SP + FS with ABS ZnNPs @ 500 ppm	55.87^a	66.27^a
T_{13}- SP + FS with ABS ZnNPs @ 1000 ppm	54.92^a	65.59^a
T_{14}- SP + FS with ABS ZnNPs @ 1500 ppm	45.00^d	58.23^c
T_{15}- RDF (100:75:50, N: P_2O_5: K_2O kg ha^{-1})	43.10^d	55.97^c
T_{16}- Control	40.65^e	52.16^d
S.Em.±	**0.50**	**0.59**

SP-Seed priming; **FS**-Foliar spraying; **BS**-Bacterial (*Pseudomonas*) synthesized; **ABS**-

actinobacterial synthesized; Seed priming @ 500 ppm common for all seed primed treatments; RDF common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.01)

Table 3. Zinc uptake of wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	**Zinc uptake (µg plant^{-1})**		
	Grain	**Straw**	**Total**
T$_{1}$- SP with BS ZnNPs @ 500 ppm	152.76^{c}	318.73^{b}	471.49^{b}
T$_{2}$- FS with BS ZnNPs @ 500 ppm	172.41^{b}	326.97^{b}	499.38^{b}
T$_{3}$- FS with BS ZnNPs @ 1000 ppm	165.87^{b}	317.39^{b}	483.26^{b}
T$_{4}$- FS with BS ZnNPs @ 1500 ppm	119.21^{d}	262.32^{c}	381.54^{c}
T$_{5}$- SP + FS with BS ZnNPs @ 500 ppm	213.79^{a}	386.99^{a}	600.79^{a}
T$_{6}$- SP + FS with BS ZnNPs @ 1000 ppm	208.35^{a}	376.08^{a}	584.44^{a}
T$_{7}$- SP + FS with BS ZnNPs @ 1500 ppm	125.78^{d}	269.09^{c}	394.86^{c}
T$_{8}$- SP with ABS ZnNPs @ 500 ppm	158.34^{c}	322.35^{b}	480.69^{b}
T$_{9}$- FS with ABS ZnNPs @ 500 ppm	177.51^{b}	332.86^{b}	510.37^{b}
T$_{10}$- FS with ABS ZnNPs @ 1000 ppm	173.30^{b}	320.60^{b}	493.89^{b}
T$_{11}$- FS with ABS ZnNPs @ 1500 ppm	121.46^{c}	266.06^{c}	387.52^{c}
T$_{12}$- SP + FS with ABS ZnNPs @ 500 ppm	220.69^{a}	395.63^{a}	616.32^{a}
T$_{13}$- SP + FS with ABS ZnNPs @ 1000 ppm	212.54^{a}	383.70^{a}	596.24^{a}
T$_{14}$- SP + FS with ABS ZnNPs @ 1500 ppm	126.90^{d}	273.68^{c}	400.58^{c}
T$_{15}$- RDF (100:75:50, N: P_2O_5: K_2O kg ha^{-1})	118.09^{d}	258.02^{c}	376.12^{c}
T$_{16}$- Control	50.81^{e}	118.92^{d}	169.74^{d}
S.Em.±	**3.24**	**7.11**	**11.82**

SP-Seed priming; **FS**-Foliar spraying; **BS**-Bacterial (*Pseudomonas*) synthesized; **ABS**-actinobacterial synthesized; Seed priming @ 500 ppm common for all seed primed treatments; RDF common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.01)

Table 4. Nutrient uptake of wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	**Nutrient uptake (mg plant^{-1})**		
	N	**P**	**K**
T$_1$- SP with BS ZnNPs @ 500 ppm	88.18^{b}	22.46^{b}	80.37^{b}
T$_2$- FS with BS ZnNPs @ 500 ppm	93.81^{b}	23.13^{b}	83.20^{b}
T$_3$- FS with BS ZnNPs @ 1000 ppm	91.34^{b}	22.42^{b}	81.38^{b}
T$_4$- FS with BS ZnNPs @ 1500 ppm	71.83^{c}	18.22^{c}	70.03^{c}
T$_5$- SP + FS with BS ZnNPs @ 500 ppm	114.53^{a}	28.01^{a}	95.74^{a}
T$_6$- SP + FS with BS ZnNPs @ 1000 ppm	111.48^{a}	27.40^{a}	93.17^{a}
T$_7$- SP + FS with BS ZnNPs @ 1500 ppm	74.21^{c}	18.77^{c}	71.63^{c}
T$_8$- SP with ABS ZnNPs @ 500 ppm	90.53^{b}	22.73^{b}	81.12^{b}
T$_9$- FS with ABS ZnNPs @ 500 ppm	96.20^{b}	23.48^{b}	84.59^{b}
T$_{10}$- FS with ABS ZnNPs @ 1000 ppm	93.73^{b}	22.72^{b}	82.50^{b}
T$_{11}$- FS with ABS ZnNPs @ 1500 ppm	72.96^{c}	18.36^{c}	70.42^{c}
T$_{12}$- SP + FS with ABS ZnNPs @ 500 ppm	115.98^{a}	28.34^{a}	97.55^{a}
T$_{13}$- SP + FS with ABS ZnNPs @ 1000 ppm	113.51^{a}	27.77^{a}	94.62^{a}
T$_{14}$- SP + FS with ABS ZnNPs @ 1500 ppm	74.73^{c}	18.89^{c}	72.10^{c}
T$_{15}$- RDF (100:75:50, N: P_2O_5: K_2O kg ha^{-1})	71.60^{c}	18.16^{c}	69.86^{c}
T$_{16}$- Control	27.92^{d}	7.90^{d}	32.99^{d}
S.Em.±	**3.42**	**0.74**	**1.97**

SP-Seed priming; **FS**-Foliar spraying; **BS**-Bacterial (*Pseudomonas*) synthesized; **ABS**-actinobacterial synthesized; Seed priming @ 500 ppm common for all seed primed treatments;

RDF common for all treatments except control
Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.01)

CONCLUSIONS

Nanomaterials, particularly in the form of nanofertilizers, have the ability to reduce various stress situations and increase the crop yields by an increase in photosynthesis, nitrogen metabolism, seedling development, carbohydrate and protein synthesis, and the transport of nutrients from roots to leaves. Nanoparticles are commonly synthesized using physical and chemical processes. However, the chemicals used in synthesis are dangerous and have a negative impact on the environment, where as physical techniques needs lot of energy, which is less efficient. Therefore, biological synthesis of nanomaterials is an ideal option which is economically profitable and eco friendly in nature. Seed priming followed by foliar spraying with biosynthesized zinc nanoparticles using actinobacteria recorded significantly higher zinc content and zinc uptake.

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