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Original Article

Zinc-coated urea and zinc-solubilizing microbes: synergistic strategies for improving zinc bioavailability in dry region soils

Hammad Anwar[1], Moazzam Jamil[1], Azhar Hussain[1*], Abubakar Dar[1], Maqshoof Ahmad[1], Saleh H. Salmen[2], Mohammad Javed Ansari[3], Rashid Iqbal[4,5*]

[1]Department of Soil Science, the Islamia University, Bahawalpur-63100, Pakistan
[2]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia
[3]Department of Botany, Hindu College Moradabad, Mahatma Jyotiba Phule Rohilkhand University, Bareilly, India
[4]Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan
[5]Department of Life Sciences, Western Caspian University, Baku, Azerbaijan

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**Corresponding author email:*
azharhaseen@gmail.com
rashid.iqbal@iub.edu.pk

Abstract

Zinc (Zn) is the most limiting micronutrient responsible for malnutrition. World Health Organization (WHO) reported deficiency of Zn is the 5th most significant cause of death and disease in underdeveloped world. However, 70% Pakistani soils are Zn deficient and responsible for Zn deficiency in crops. The present study aimed to mitigate Zn deficiency and improve them a nd Zn use efficiencies through synergizing dry region Zn solubilizing plant growth promoting rhizobacteria (PGPR) by coating on Zn coated urea. Pre-isolated dry region Zn solubilizing isolates were evaluated for zinc solubilization, urease activity, siderophores production, organic acid production and ACC-deaminase activity. Four effective strains *Bacillus amyloliquefaciens* (IUB-34), *Klebsiella variicola* (IUB-96), *Klebsiella variicola* (IUB-80) and *Klebsiella pneumoniae subsp. pneumoniae* (IUB-93) and their consortium coated on Zn coated urea. This improved product was tested for N and Zn release pattern, growth promotion and Zn biofortification in pot trial on wheat. Results showed that SPAD chlorophyll value, root, shoot length and their dry weight was significantly improved ($p \leq 0.05$) by 19.4, 20.3, 45.9, 27.3 and 39.5%, respectively, over control. Similarly, N, P, K, Zn, Fe in grains and 100-grain weight was significantly increased ($p \leq 0.05$) by 97.5, 23.5, 61.1, 63, 32 and 50.5%, respectively, over control. The results confirmed that dry region Zn solubilizing bacterial consortium coated on Zn coated urea is an efficient method for the biofortification of Zn in wheat grains and can effectively overcome Zn deficiency in humans.

Keywords: Zinc, Dry region, Wheat, Consortium, Zn coated urea, Biofortification

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Introduction

Micronutrients are important for productivity, growth and quality of crop at optimal level because they do their activity for appropriate growth functioning and quality development of different crop plants. Zinc is the essential micronutrient on earth and important for all living organisms (Shaikh and Saraf, 2017; Nazir et al., 2016). In plant tissues Zn exists in low concentrations of around 5 to 100 $mgkg^{-1}$ (Saravanan et al., 2004) which is important for proper working and development, quality, optimum growth and productivity of crop plants (Jha, 2019). It performs a significant role in the production of starch and chlorophyll synthesis in plants (Rehman et al., 2018), phytohormones production (gibberellins, auxins, cytokinins and absicic acid) (Imran et al., 2014), carbohydrates and N metabolism as well as resistance against oxidative damage or oxidation (Potarzycki and Grzebisz, 2009; Cakmak, 2008). Photosynthesis, pollens formation, cell membrane integrity, resistance against diseases (Gurmani et al., 2012), antioxidant enzymes levels (Sbartai et al., 2011) and reactions of energy transfer (Broadley et al., 2012) are also performed by Zn. It also plays a positive role in the water uptake regulation and decline the toxic effect of salt and heat stress on crop plant (Peck and McDonald, 2010). According to WHO (2012), deficiency of Zn is the main reason of disease and death in human because they mostly depend on cereals like wheat, maize and rice for their routine caloric intake. The vital biological activities, such as structural, regulatory and catalytic functions in the body of human beings, need to be a suitable amount of Zn contents for appropriate functioning (Mohammadi et al., 2021).

In Pakistan application of essential nutrients always restricted to N (nitrogen), P (phosphorus) and K (potassium). In Pakistan, due to alkaline-calcareous soils, Zn is the most deficient micronutrient (Rashid and Ryan, 2008). In semi-arid to arid re gions, amount of Zn in soil is low due to different factors of soil (Imran et al., 2014). In Pakistan almost 37% population is affected by Zn malnutrition (UNDP, 2003). Living organisms require Zn in optimum level for the healthy development and growth (Huang et al., 2022). Micronutrients deficiency exists in most cereals' crops, which can affect worldwide over 2 billion population (Waqeel and Khan, 2022). Wheat holds significant importance as a staple food crop in Pakistan (Mirza et al., 2015). Wheat serves as a primary cereal crop, contains valuable nutrition, especially for the countries which have limited resources (Yaqoob et al., 2022). Due to its taste and nutritional value, wheat in food crops is the most popular like vitamins, proteins and calories (Ahmad et al., 2022).

Keeping in view, the importance of Zn for plants and humans, different methods are being used to improve the Zn bio-availability in soil and its further utilization by crops, like fortification (addition of require nutrient in different food items), food diversification/modification (food processing/cooking according to nutritional demand), supplementation (clinical nutrients of nutrients) and biofortification in which bioavailable nutrient elements improvement in edible part of plants (Mayer, 2008). It is a sustainable and economical method to minimize the problem of low Zn concentration in food crops (Bouis and Welch, 2010).

In human beings, biofortification is the ultimate way to decrease the Zn deficiency and enhance the soil-crop-human inter-relationship (De Valença et al., 2017). The method of biofortification aims to increase the minerals like Zn, Fe (iron), I (iodine), Se (selenium) etc. in grains, so that people that consume such grains improve the mineral intake (De Valença et al., 2017). In low-income areas malnutrition of micronutrient or hidden hunger is highly prevalent due to their less purchasing power and ultimately, they can't afford supplements or a healthier diet that contain micronutrients. The most sustainable method for alleviating the micronutrient deficiency in these conditions is to improve the nutritional value of the widely consumed staple food grains (Cakmak, 2008). An efficient biofortification technique should confirm that yield of grains increase or at least maintained, improve the Zn contents in grains for improving the benefits of human health, and stable grain performance across the environments (Zou et al., 2012). An encouraging and innovative technique for biofortification of zinc is the use of plant growth promoting rhizobacteria; a particular type of bacteria which could solubilize insoluble or fixed amount of soil Zn and accessible for plants (Mumtaz et al., 2017; Imran et al., 2014). The rhizobacterial strains have the capability to release 2-ketogluconic acid (Fasim et al., 2002), gluconic acid, 5-ketogluconic acid (Saravanan et al., 2007), chelating ligands, secrete amino acids, vitamins and phytohormones (Saravanan et al., 2004), these compounds can solubilize fixed or insoluble forms of Zn. Different PGPR inoculation in plants performed increased Zn contents and growth. These

inoculants are *Bacillus subtilis* (Samaras et al., 2022) *Bacillus aryabhattai* (Prathap et al., 2022) and *Rhizobium* and *Pseudomonas* (Naz et al., 2016). Among bacterial isolates to shown solubilization of Zn on laboratory stage are *Serratia* sp., *Burkholderia* sp., *Klebsiella* sp., *Gluconacetobacter* sp., *Acinetobacter* sp., (Haroon et al., 2022), *Pseudomonas* spp., (Hashemnejad et al., 2021), *Enterobacter* sp., and *Citrobacter* sp. (Ajmal et al., 2021).

Keeping in view, the Zn deficiency in soil and less amount of Zn in food crops like cereals, because they are important staple diets in Pakistan, a study was planned to obtain the following objectives, to determine the effect of dry region Zn solubilizing strain coated on Zn coated urea, on temporal release of N and Zn in soil under axenic conditions. Also evaluate the effect of Zn solubilizing strains of dry region coated on Zn-coated urea on growth, biofortification and yield of wheat in pot condition.

Material and Methods

Characterization of Zn solubilizing PGPR

Dry region rhizobacterial strains IUB-34, IUB-80, IUB-93, IUB-96 was taken from Soil Microbiology and Biotechnology Laboratory, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur. The PGPR strains were characterized for the solubilization of Zn, siderophore production, urease activity, ACC-deaminase production. Qualitative Zn solubilization ability of bacteria were assessed by using Bunt and Rovira media with the addition of 0.1% ZnO as a Zn source to examine the capability of rhizobacterial strains to solubilize ZnO (Bunt and Rovira, 1955). The amount of solubilizing of each particular PGPR isolates was checked by calculating the solubilization zone around the bacterial colonies according to Saravanan et al. (2004). For the determination of Zn quantitatively, broth culture using Bunt and Rovira medium were prepared with the addition of 0.1% ZnO and adjust to pH 7.0. The concentration of solubilized Zn was obtained by using atomic absorption spectrophotometer and comparing the Zn concentrations in inoculated sample with un-inoculated control in mg L^{-1} (Saravanan et al., 2004). The Chrome Azurol S (CAS) agar media was used for the determination of the siderophore production ability of PGPR strains (Gopalakrishnan et al., 2011). Supplementation of 3 mM ACC, $(NH_4)_2SO_4$ and $MgSO_4$ in minimal medium (Dworkin and Foster, 1958) was used to check the activity of ACC-deaminase in PGPR strains as proposed by Penrose and Glick (2003). For the determination of urease activity, the pre-isolated PGPR strains was inoculated in Christensen's urea-broth media and incubated at 30±2°C for 24 hours. The presence of pink color in growth medium will consider urease positive (Cappuccino and Sherman, 2005).

Determination of microbially produced organic acids

The Zn solubilizing bacterial strains of dry region IUB-34, IUB-80, IUB-93, IUB-96 was inoculated in the test tubes which have 50 mL of LB broth media with and without ZnO and incubated at 28ºC for 48 h. Each sample after incubation was centrifuged at 4 ºC and 10,000rpm and supernatant were collected separately. The metabolites in the supernatant were extracted three times in methanol (HPLC grade) in 1:1 ratio by the use of separating funnel. Organic acids (ferulic acid, cinamic acid, chlorggenic acid, caffeic acid, gallic acid and syrirgic acid) was detected from the extracted samples using HPLC (Butsat et al., 2009).

Compatibility test

Zinc solubilizing bacterial isolates was cross streaked on agar plates of LB media and incubated at 30 ± 1 °C for 48h. At the point of intersection, compatible isolates were showing no zone of inhibition at the point of intersection (Fukui et al., 1994).

Identification of bacterial strains through 16S rRNA Gene Sequencing

The pre-isolated Zn solubilizer, ACC-deaminase-producing, siderophores producing and urease producing strains were determined for molecular identification by partial gene sequence of 16S rRNA. The first step is, DNA extraction that were carry out from strains culture by using a proteinase K containing extraction buffer (Mahuku, 2004). Extraction of DNA was carried out by a polymerase chain reaction (PCR) to amplify the 16S rRNA for sequencing by 1492R and 27F universal primer pairs. The reaction material contained 3.7 µL of crude DNA, 3 µL of 1492R (10 µM), 3 µL of 27F (10 µM), and 7.5 µL of H_2O. The situations for thermal cycling were 1 cycle for 1 min at 95°C, followed by 94°C, for 1 min 32 amplification cycles and at 55°C 20s cycle of hold. The products obtained after PCR were send to the commercial-service for 16S rRNA sequencing, Macrogen® Seoul, Korea. The sequences of nucleotide obtained from

Macrogen were BLAST to compare with the available NCBI genome database. According to the Thompson et al. (1997) procedure, a phylogenetic tree was created on MEGA-X software. According to neighborhood joining method NJ Plot was used to process the data (Perrière and Gouy, 1996).

Formulation of bacterial culture for coating on Zn coated urea

The broth-culture was prepared by using pre-isolated strains in conical flask (1000 ml) containing the media described by Bunt and Rovira (1955). The flasks after inoculation were incubated at 28 ± 1°C for 48 h at 100 rpm in shaking incubator. Before use, dilute the cell culture to maintain the homogeneous cell density and achieved the optical density of 0.5 at 535 nm [10^8 –10^9 cfu (colony forming units)/mL]. The bacterial consortium was made by equally mixing the homogenous culture of four strains in 1:1:1:1 ratio and further single strain and consortium were applied at 3% v/w on urea for effective coating. Zn coated urea with dry region zinc solubilizing bacteria was formulated by the standard procedure adopted for BNNF (Zabardast Urea) producer company First Biotech LLC Lahore.

Determination of zinc and bacterial population in coated fertilizer

Available Zn present in soil/fertilizer was measured by extraction procedure of ammonium-bicarbonate DTPA described by Soltanpour and Schwab (1977), and later revised by Soltanpour and Workman (1979) on atomic absorption spectrophotometer. Total Zn analysis from soil/fertilizer sample, mixed 0.2-gram sample with 6 mL concentrated sulphuric acid in 100 ml conical flask and placed in standard room-temperature for overnight. After that add 1 ml hydrogen per oxide into flask and heated on hot plate for 1 hrs at 300 °C. Last step was performed again and again till the milkish white or transparent color appearance. After that dilute the sample up to the mark and performed further analyses. The Zn was determined on atomic absorption spectrophotometer (Model: 240 FS, Agilent Technologies, USA) by the using of standard procedure defined by Ryan et al. (2001). For microbial population take 1 gram soil/fertilizer sample and perform standard pour plate or serial dilution technique was used to examine the population of bacteria in terms of CFU (colony-forming units) (Alexander, 1983).

Release pattern of Zn and N

A pot/jar trial was conducted at the growth room of Department of Soil Science to investigate the rate of Zn and N release form the final product with different interval of time. For this experiment soil was taken from the research farm area of Department of Soil Science, The Islamia University of Bahawalpur. Before conducting the experiment, soil analysis was performed.

Pre-sowing soil analysis

Bouyoucos hydrometer method was used to determine the fractions of sand, clay and silt in the soil samples (Moodie et al., 1959). However, the textural class was examined by the use of USDA Texture Triangle online. From saturated soil paste ECe and pH was determined by using the method of 21a defined in U.S. Salinity Laboratory Staff (1954) by the use of EC and pH meter. Soil organic matter was determined, as per standard procedure proposed by Walkley and Black, (1934) through oxidation with potassium di chromate and titration with 0.5 M Ferrous Ammonium Sulfate solution. For total N measurement sample was digested in H_2SO_4 according to the method described by Hibbard's and Ginning and distillation was done in Kjeldhal's digestion and distillation apparatus (Jackson, 1962).

Table-1. Physio-chemical parameters of soil before sowing

Characteristics	Unit	Values
Clay	%	15.5
Silt	%	38.6
Sand	%	46.2
Textural Class	--	Loam
Ece	(dS m^{-1})	1.3
pHs	--	7.8
Saturation Percentage	%	42
Nitrogen	%	0.02
Available Potassium	mg kg^{-1}	105
Available Phosphorus	mg kg^{-1}	5.1
Extractable Zn	mg kg^{-1}	0.6
Iron	mg kg^{-1}	0.65
Organic Matter	%	0.56

Watanabe and Olsen, (1965), method was performed for the detection of extractable phosphorus and reading were taken in spectrophotometer (Model G6860A, Agilent Technologies Cary 60 UV-Vis, Australia). Extraction of soil was done with 1 N ammonium-acetate solution and extractable potassium was measured by using Flame-Photometer (Model: BWB-XP, BWP Technologies, Uk) (Method 1la, Salinity Laboratory Staff, 1954). To measure the soil urease activity, a soil sample was prepared according to the method described by Alef and Nannipieri (1995) and optical density was measured. Pre-sowing soil analysis data was shown in Table 1.

Pot trial

A pot study was carried out to assess the effectiveness of microbially impregnated Zn coated urea with respective isolates. For this purpose, wheat was sown in pots in their respective growing season and coated fertilizers were applied as compare with market product of Zn coated urea and urea+$ZnSO_4$ supplementation. The recommended fertilizer doses P and K were applied before sowing and all other agronomic practices were followed until harvest. Data was recorded at maturity for growth parameters and at harvest for yield and quality parameters.

Treatment plan for pot trial

T0	Control	**T4**	IUB-80 (Coated on Zn coated Urea)
T1	Simple Urea + $ZnSO_4$	**T5**	IUB-96 (Coated on Zn coated Urea)
T2	BNFF Urea (Zabardast Urea)	**T6**	IUB-93 (Coated on Zn coated Urea)
T3	IUB-34 (Coated on Zn coated Urea)	**T7**	Consortium (Coated on Zn coated Urea)

Plant analysis

To examine the physiological characters, plants are analyzed when physiological maturity was achieved (chlorophyll SPAD value). While the growth parameters were examined at the plant maturity and also data regarding yield parameters was conducted. For analysis of mineral, take 0.2 g dry homogeneous plant sample and mixed in 100 ml conical flask with 6 mL concentrated sulfuric acid and at the room temperature placed for overnight. After that add the 1 mL H_2O_2 in the flask and placed on hot plate for heating for 1 hour at 300 °C. Repeat this step again and again till the presence of milky white or transparent color. After that dilute the sample up to the mark in the conical flask and store it for further analysis. Measure the macro and micronutrients N, P, K, and Zn, Fe using standard procedure described by Ryan et al. (2001).

Statistical analysis

The data collected from this research was analysed by statistically using CRD (completely randomized design). However, the treatment means was computed through Tucky's test (HSD) for significant differences among treatments by using Statistix v. 8.1 (Analytical Software, Tallahassee, FL, USA) (Steel et al., 1997).

Results

Characterization of pre-isolated zinc solubilizing isolates

Zinc solubilizing isolates of dry region were characterized for zinc solubilization qualitatively and quantitatively, siderophore production, ACC-deaminase, urease production. Results of Table 2 and 3 indicate that all the isolates taken from Soil Microbiology and Biotechnology Laboratory, Department of Soil Science, The Islamia University of Bahawalpur, Pakistan were positive against zinc solubilization. While the isolate IUB-34 shows the maximum halo zone diameter that was 21.3 mm, however the maximum solubilization efficiency, solubilizing concentration and solubilizing index were shows the isolate IUB-96 that is 240.3%, 26 mgL^{-1}, 3.6 respectively. While the isolates IUB-80, IUB-93 also showed effective results in Zn solubilization. These isolates (IUB-34, IUB-80, IUB-96, IUB-93) also showed positive results in production of siderophore, ACC-deaminase activity and urease activity. These selected isolates were further used for evaluation.

Organic acids production

Results of dry region Zn solubilizing isolates showed different patterns in the chromatogram of rhizobacterial metabolites and different peaks. In Zn solubilizing metabolites identified the different compounds such as malic acid, acetic acid, citric acid, succinic acid, gibberallic acid, gluconic acid and oxalic acid (Table 4). All rhizobacterial isolates produce citric acid, gibberallic acid, gluconic acid and oxalic acid. The highest amount of oxalic acid was measured in the metabolite of *Bacillus amyloliquefaciens* IUB-34 (16 µg mL^{-1}), followed by *Klebsiella pneumoniae* IUB-93 (12.5 µg mL^{-1}), *Klebsiella variicola* IUB-96 (11 µg mL^{-1}) and *Klebsiella* sp. IUB-80 (7 µg mL^{-1}). *Klebsiella pneumoniae* IUB-93 synthesis all organic acids such as

acetic acid (1.09 µg mL^{-1}), malic acid (0.34 µg mL^{-1}), succinic acid (0.41 µg mL^{-1}), citric acid (5.4 µg mL^{-1}), gibberallic acid (1.98 µg mL^{-1}), gluconic acid (0.63 µg mL^{-1}) and oxalic acid (12.5 µg mL^{-1}). Malic and succinic acids were not detected in the metabolites of *Klebsiella* sp. IUB-80 and acetic and succinic acids were not recorded in the metabolites of *Bacillus amyloliquefaciens* IUB-34.

Table-2. Effect of rhizobacterial isolates on solubilization of zinc, siderophore production, ACC-deaminase and urease production (n=3).

Bacterial Isolates	Solubilization of Zinc	Siderophore Production	ACC-Deaminase	Urease Production
IUB-34	+++	++	++	+++
IUB-80	+++	+++	+++	+++
IUB-93	++	+++	+++	+++
IUB-96	+++	++	+++	++

The symbol (+++) shows significant activity and (++) shows less significant or good traits.

Table-3. Qualitative and quantitative zinc solubilization of dry region zinc solubilizing isolates (n=3).

Bacterial Isolates	Solubilization of Zinc				
	SI	CD (mm)	HZD (mm)	SC (mgL^{-1})	SE (%)
IUB-34	3.6 a ± 0.05	8.2 b ±0.01	21.3 ab ±0.29	24.7 b ±0.29	259.8 c ±0.50
IUB-80	3.6 a ±0.08	7.5 d ±0.02	19.7 b ±0.58	24 c ±0.50	262.7 b ±0.50
IUB-93	3.6 a ±0.05	8.4 a ±0.01	21.7 a ±0.29	26 a ±0.87	258.3 c ±0.30
IUB-96	3.7 a ±0.04	7.8 c ±0.01	20.7 ab ±0.76	25 b ±0.50	265.4 a ±0.01
LSD ($p \leq 0.05$)	0.2123	0.0414	1.9597	2.1741	1.4445

Data shown the means of three replicates; same letter(s) in the means within the column not different significantly according to the (LSD) test at $p \leq 0.05$; SI = solubilization index; CD= colony diameter HZD = halo zone diameter; SC = solubilized concentration; SE = solubilization efficiency.

Table-4. Determination of organic acids by using HPLC (n=3).

Organic Acids	Dry Region Zinc Solubilizing Strains			
	Bacillus amyloliquefaciens IUB-34	*Klebsiella* sp. IUB-80	*Klebsiella variicola* IUB-96	*Klebsiella pneumoniae* IUB-93
	(µg/mL)			
Malic acid	1.1 d	ND	ND	0.34 f
Acetic acid	ND	0.86 c	0.77 e	1.09 d
Citric acid	3.4 c	2.2 b	4.8 c	5.4 b
Succinic acid	ND	ND	1.05 d	0.41 f
Gibberellic acid	5.2 b	0.54 d	8.3 b	1.98 c
Gluconic acid	0.99 e	0.39 e	0.68 f	0.63 e
Oxalic acid	16 a	7 a	11 a	12.5 a

ND = Not detected

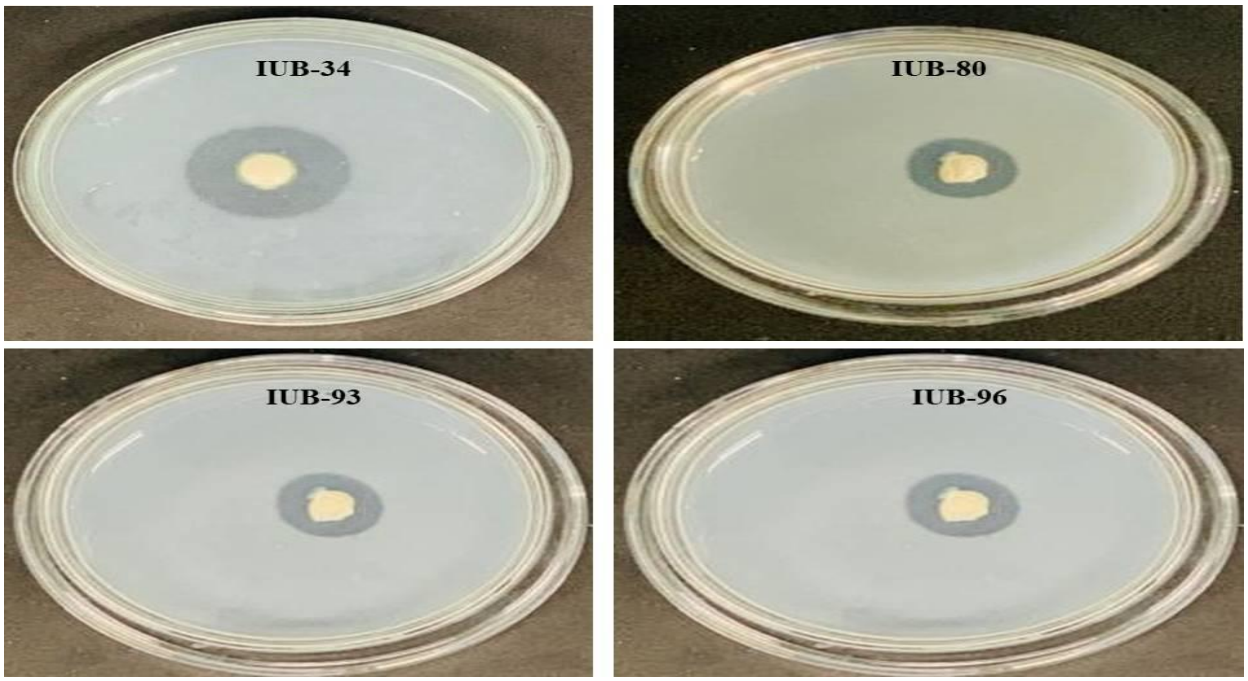


Figure-1. Zinc solubilization due to dry region rhizobacterial isolates of IUB-34, IUB-80, IUB-93 and IUB-96 (Halo zone around the colony shows the zinc solubilization)

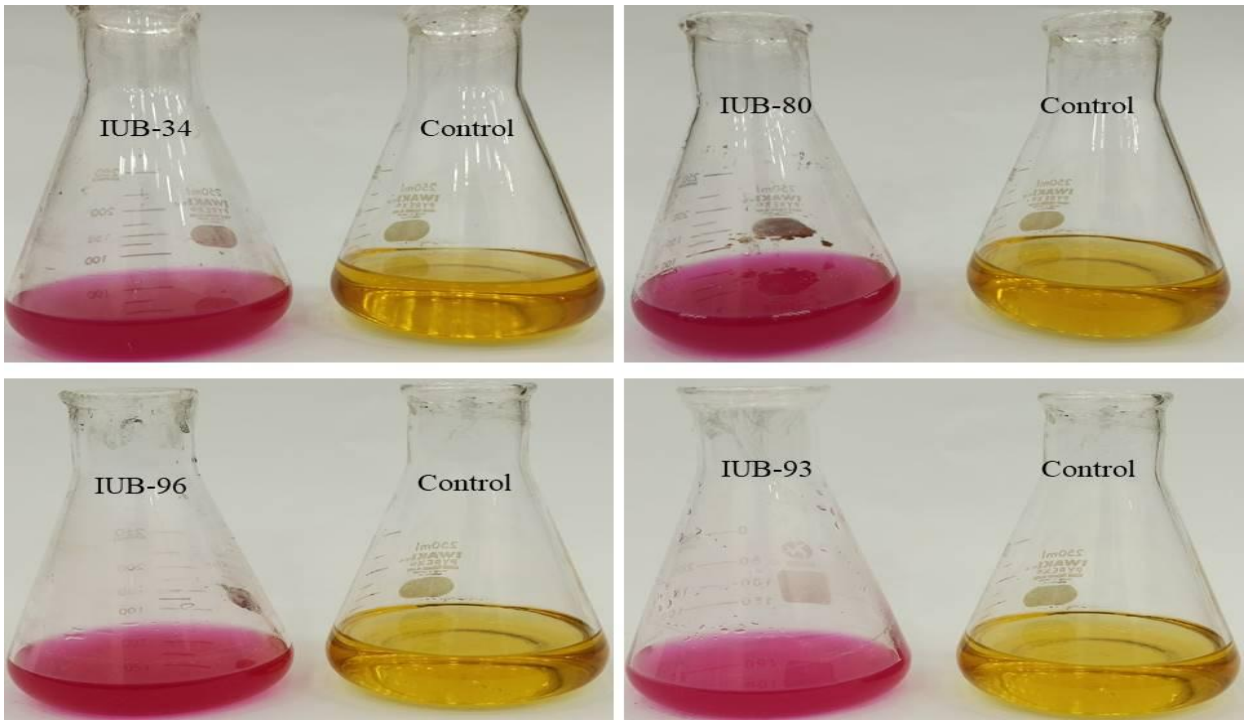


Figure-2. Urease activity due to dry region rhizobacterial isolates of IUB-34, IUB-80, IUB-93 and IUB-96

Compatibility test

The selected zinc solubilizing bacterial isolates of dry region was cross streaked on agar plate media to check the compatibility of isolates. Results confirm that all the selected four isolates of zinc solubilizing bacteria of dry region (IUB-34, IUB-80, IUB-96, IUB-93) were compatible with each other. Further these compatible isolates prepared for coating on zinc coated urea to assess the growth promotion and bio-fortification of zinc in wheat.

Identification of dry region zinc solubilizing isolates

Identification of pre-isolated zinc solubilizing isolates of dry region (IUB-34, IUB-80, IUB-96, IUB-93) were through partial gene sequence of 16S rRNA (Figure 3). The bacterial isolate IUB-34 was 100% resembled *Bacillus amyloliquefaciens* sp. and the isolate were identified as *Bacillus amyloliquefaciens* sp. IUB-34 and further submitted to NCBI under the accession number ON936039. The rhizobacterial isolates IUB-80 and IUB-96 was 98% similar with *Klebsiella variicola* sp. and these isolates were identified as *Klebsiella variicola* sp. IUB-80 and *Klebsiella variicola* sp. IUB-96 and submit to NCBI with accession numbers ON936042 and ON936040 respectively. IUB-93 isolate were similar to *Klebsiella pneumoniae subsp. Pneumonia* sp. and their similarity is 95%. This isolate is identified as *Klebsiella pneumoniae subsp. Pneumonia* sp. IUB-93 and submitted to NCBI with accession number ON936041.

Determination of bacterial population and zinc in coated fertilizer

Data shown in Figure 4 (A and B) represent the bacterial population and Zn contents in Zn coated urea fertilizer coated with different dry region Zn solubilizing isolates and their consortium as compare with already develop product of BNFF Urea Z© (Zabardast Urea). Maximum bacterial population, available and total Zn contents was recorded in T5 treatment where bacterial consortium of four isolates was coated on zinc coated fertilizer which contains 49 $\times 10^4$, 1.01% and 1.33% respectively, over un-inoculated control. Further increase in population, available and total zinc was observed in T1 (IUB-34), T2 (IUB-80), T3 (IUB-96) and T4 (IUB-93) treatment which contain (44 $\times 10^4$, 0.97%, 1.3%), (41 $\times 10^4$, 0.94%, 1.29%), (42 $\times 10^4$, 0.92%, 1.29%), (38 $\times 10^4$, 0.91%, 1.29%) respectively. While in BNFF Urea Z© (Zabardast Urea) bacterial population and available and total zinc was (42 $\times 10^4$, 0.8%, 1.28%) observed.

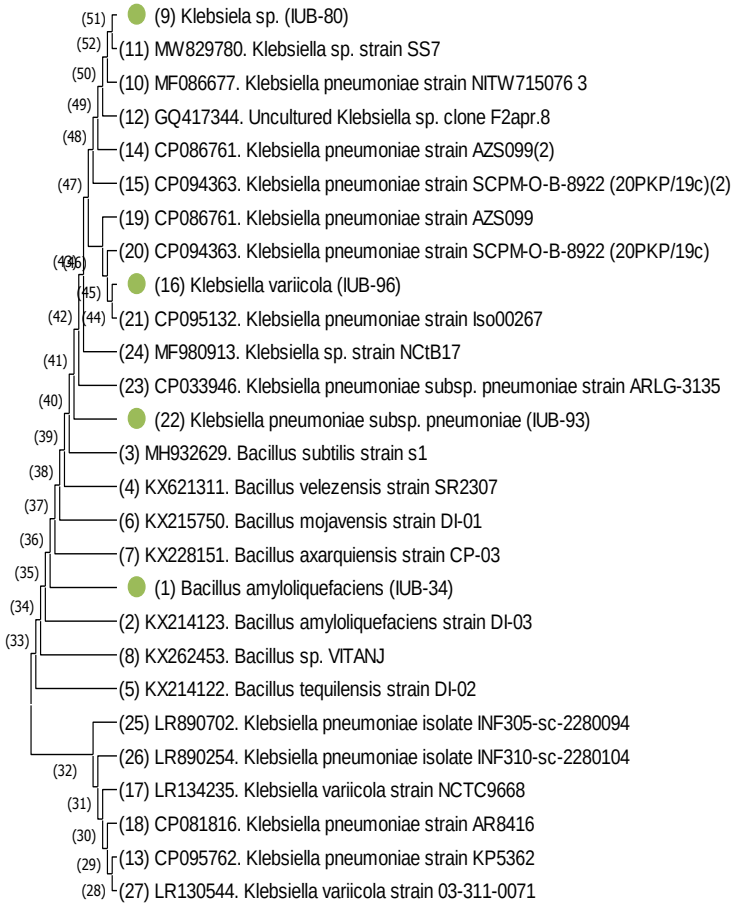


Figure-3. Phylogenetic tree of the bacterial isolates *Bacillus amyloliquefaciens* (IUB-34), *Klebsiella variicola* (IUB-96), *Klebsiella variicola* (IUB-80), *Klebsiella pneumoniae subsp. pneumoniae* (IUB-93) (accession number: ON936039, ON936042, ON936040, ON936041).

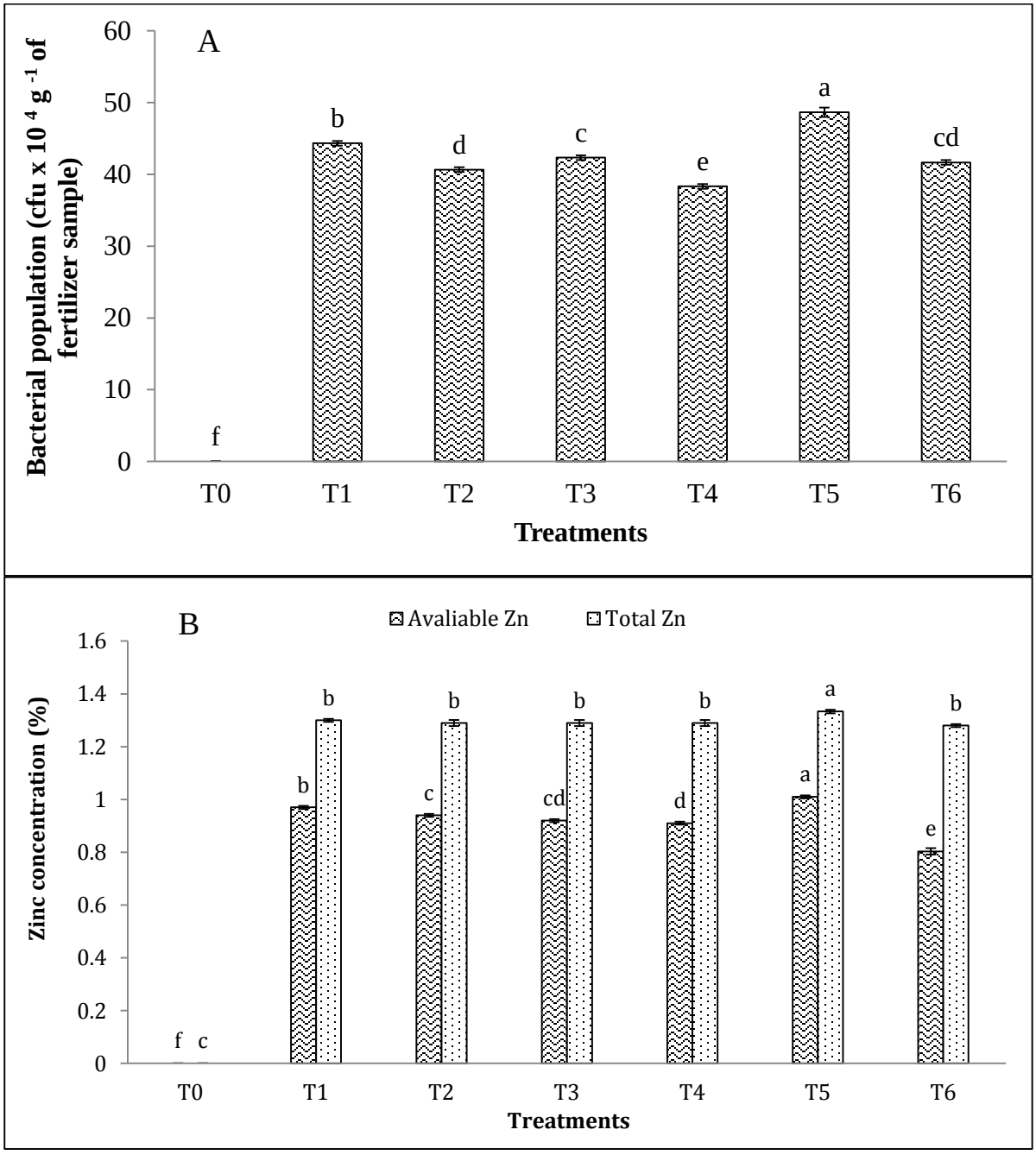


Figure-4. Effect of different dry region zinc solubilizing isolates coated on Zn coated urea on (A) bacterial population, (B) available and total zinc contents in coated fertilizer, n = 3. Bars share similar letter(s) were not vary from each other at p≤0.05 statistically.

Release of zinc and urease activity in soil at different interval of time

Figure 5 (A, B) confirms zinc release pattern and urease activity in soil with different interval of time in different treatments. Results confirm that all applied treatments presenting an increasing trend of zinc release and urease activity over control where no zinc was used. But highest and constant improvement was observed in treatment containing microbial consortium coating on Zn coated urea (T7). A gradual increase in zinc concentration from 0.77 mg kg^{-1} and 7.4 mg NH_4-N kg^{-1} h^{-1} urease activity at the 1st day of

study to 2.03 mg kg^{-1} of zinc and 56.3 mg NH_4-N kg^{-1} h^{-1} urease activity after 75 days was recorded under T7 treatment which was 165% more than the soil Zn contents and 604% more than urease activity at the start of the incubation.

While the treatment with already developed product BNFF Urea Z© (Zabardast Urea) was applied (T2) also showed increasing Zn content after 45th days of incubation and reached 1.5 mg kg^{-1} Zn at the last day of incubation study. But in urease activity treatment T2 shows decline in urease activity and reached 26.3 mg NH_4-N kg_{-1} h^{-1} at the end of study. However, the treatment where only Urea+$ZnSO_4$ (T1) was used showed enhance in the concentration of zinc and urease activity at the 30th days of study, but after that immediate shows decline in Zn and urease activity as compare with microbial and Zn coated urea.

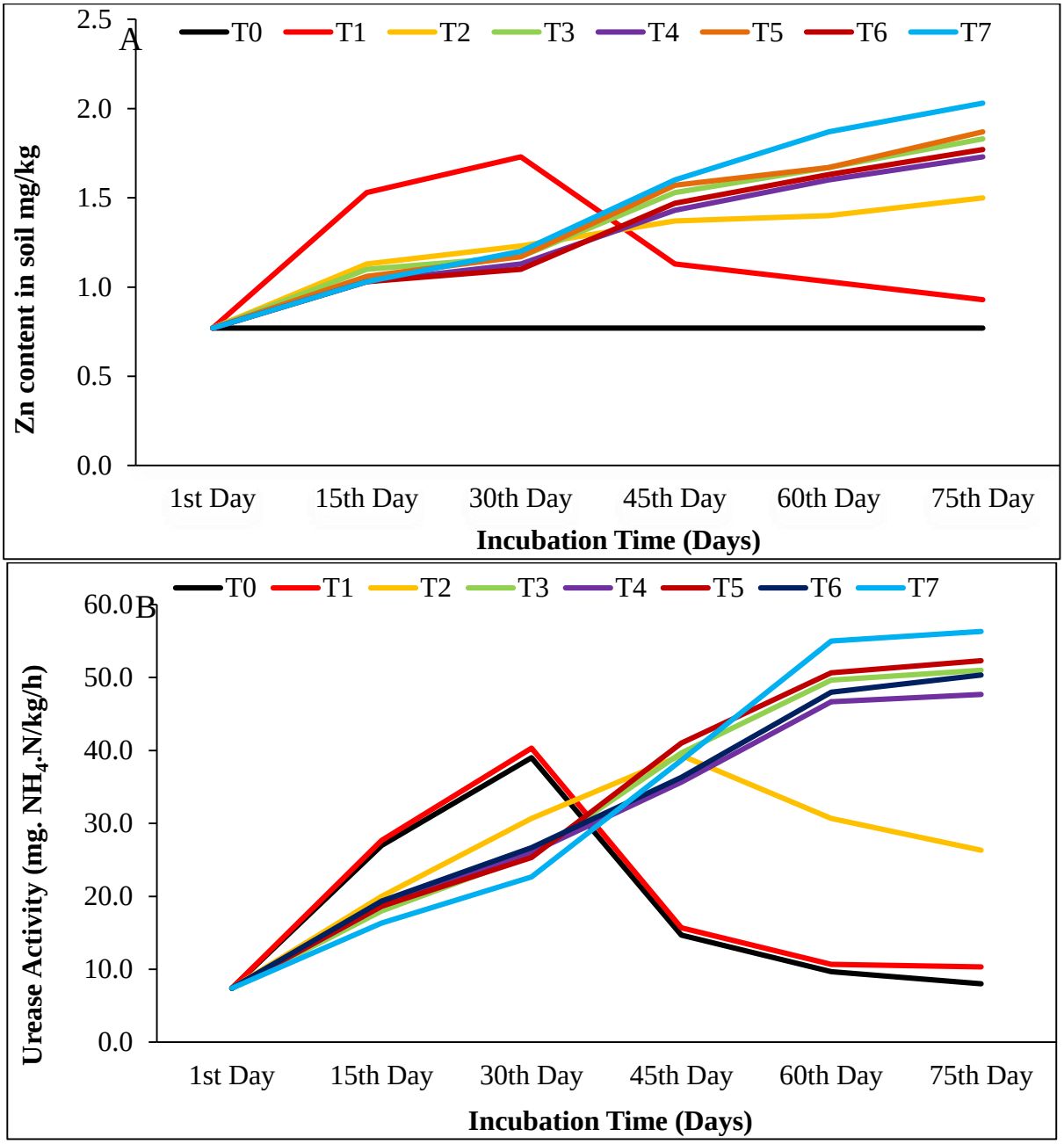


Figure-5. Effect of different treatments of dry region zinc solubilizing isolates coated on Zn coated urea on (A) release of Zn and (B) urease activity in soil with different interval of time n = 3.

Pot trial wheat

Growth parameters

In pot trial of wheat, results regarding growth parameters confirmed that the use of different bacterial isolates of dry region and their consortium coated on Zn coated urea along with already develop product BNFF Urea Z© (Zabardast Urea) and zinc sulphate, enhance the shoot length, root length, shoot dry weight, root dry weight and spike length over control (Table 5). However, shoot length, root length and their dry weight of wheat was only increase when the application of zinc coated fertilizer coated with dry region Zn solubilizing isolates and their consortium was done. Notably, the maximum improvement in root length, shoot length and their dry weight was observe in treatment contain consortium coated on Zn coated urea and these are 20.3, 45.9, 27.3 and 39.5% over control. While spike length of wheat was also improved with the application of consortium coated on Zn coated urea and showed 33% over control where no Zn source was applied.

SPAD chlorophyll value

The effectiveness of different bacterial isolates of dry region and their consortium coated on zinc coated urea along with already develop product BNFF Urea Z© (Zabardast Urea) and zinc sulphate on the chlorophyll SPAD value of wheat plant are shown in (Table 5). Significant increase was observed in chlorophyll contents was seen in treatment which contains consortium coating on zinc coated urea that is 19.4% over control were no zinc was used. While the treatment which contain BNFF Urea Z© (Zabardast Urea) also shows better results as compared to control that is 8.1% improvement in chlorophyll contents.

100-grain weight

Significant improvement in the 100-grain weight was observed in wheat crop as a result of the application of the treatments containing different bacterial isolates consortium from dry region coated on zinc coated urea along with BNFF Urea Z© (Zabardast Urea) and zinc sulphate (Figure 6). The highest improvement in grain weight was observed in treatment which contains consortium coating on zinc coated urea that is 50.5% as compare to control. Whereas treatment where BNFF Urea Z© (Zabardast Urea) were applied also shows better results as compared to control that is 11.8% increase in grain weight.

N, P, K in wheat grains

Use of dry region zinc solubilizing isolates coating on zinc coated urea along with zabardast urea and zinc sulphate on wheat crop performed significant improvement in N, P, K contents in wheat seeds as compared to control where no zinc source was used (Figure 7 A, B and C). Use of Zn coated urea coated with dry region zinc solubilizing isolates enhance the N contents up-to 97.5%, P contents up to 23.5% and K contents up to 61.1% in wheat grains, over control. Already develop product BNFF Urea Z© (Zabardast Urea) also improve the N, P, K contents in wheat grains that shows 27.5, 5.1 and 17.7% respectively, over control.

Table-5. Effect of different treatments of Zn coated urea on shoot and root length and their dry weight, spike length and SPAD value of wheat, n = 3.

Treatment	Shoot length	Shoot Dry Weight	Root Length	Root Dry Weight	Spike Length	SPAD Value
T0	40.7 g	7.9 e	13.1 g	4.3 e	7.4 e	32.9 g
T1	42.3 f	8.2 e	13.4 g	4.4 e	7.5 e	33.9 f
T2	44.7 e	8.8 d	14.0 f	4.6 d	8.2 d	35.6 e
T3	54.3 b	10.3 b	15.4 b	5.2 b	9.3 b	38.4 b
T4	50.3 c	9.4 c	14.7 d	4.9 c	8.7 c	36.6 d
T5	51.7 c	10.0 b	15.0 c	5.1 b	9.1 b	37.6 c
T6	48.3 d	9.1 d	14.3 e	4.8 cd	8.6 c	36.2 d
T7	59.3 a	11.1 a	15.8 a	5.4 a	9.8 a	39.3 a
LSD ($p \leq 0.05$)	**1.5801**	**0.3533**	**0.2892**	**0.1413**	**0.3638**	**0.5347**

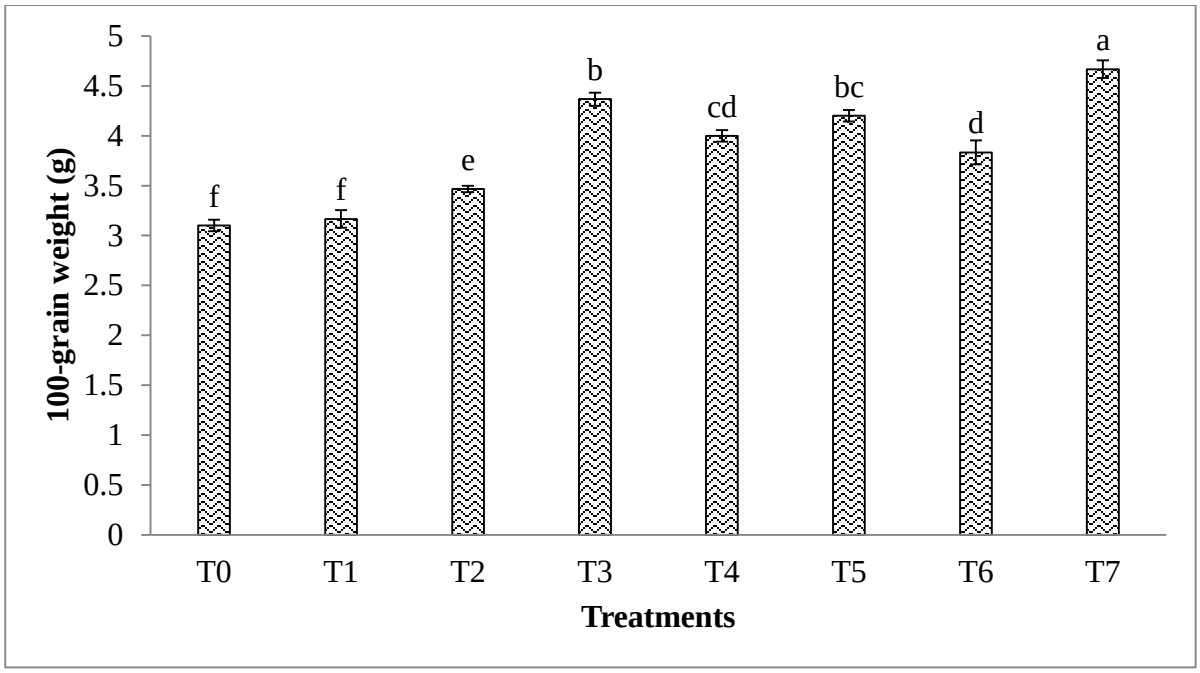
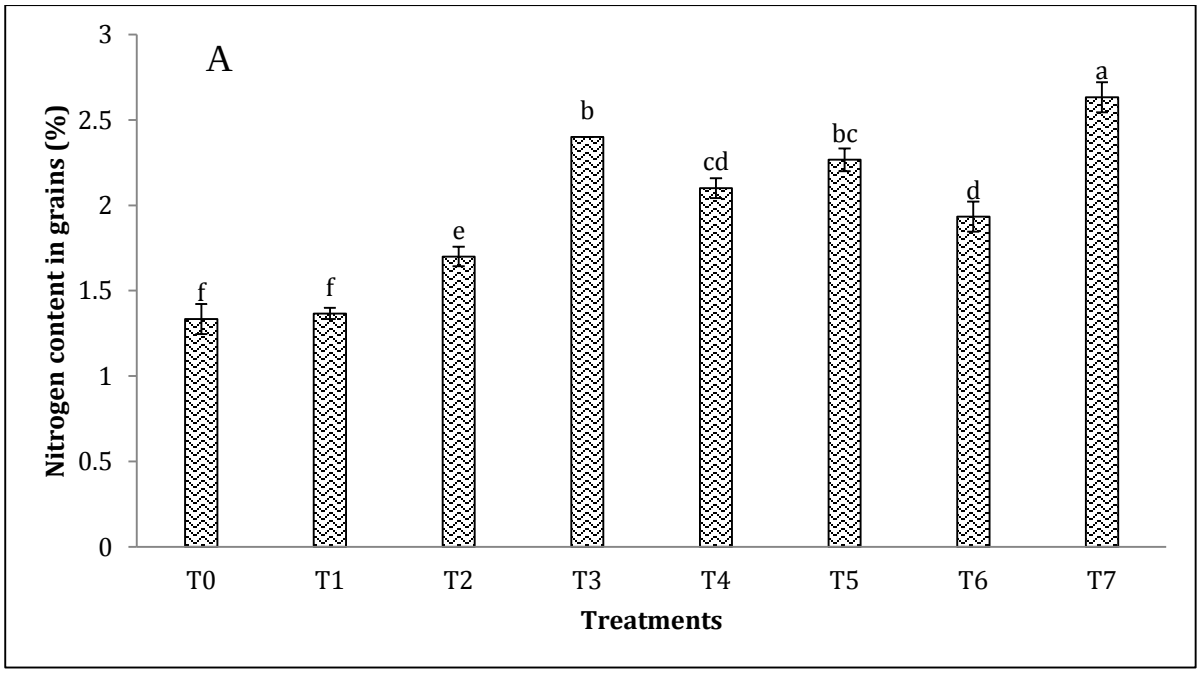


Figure-6. Effect of different dry region zinc solubilizing isolates coated on Zn coated urea on 100-grain weight (g) n = 3. Bars share similar letter(s) were not vary from each other at p≤0.05 statistically.



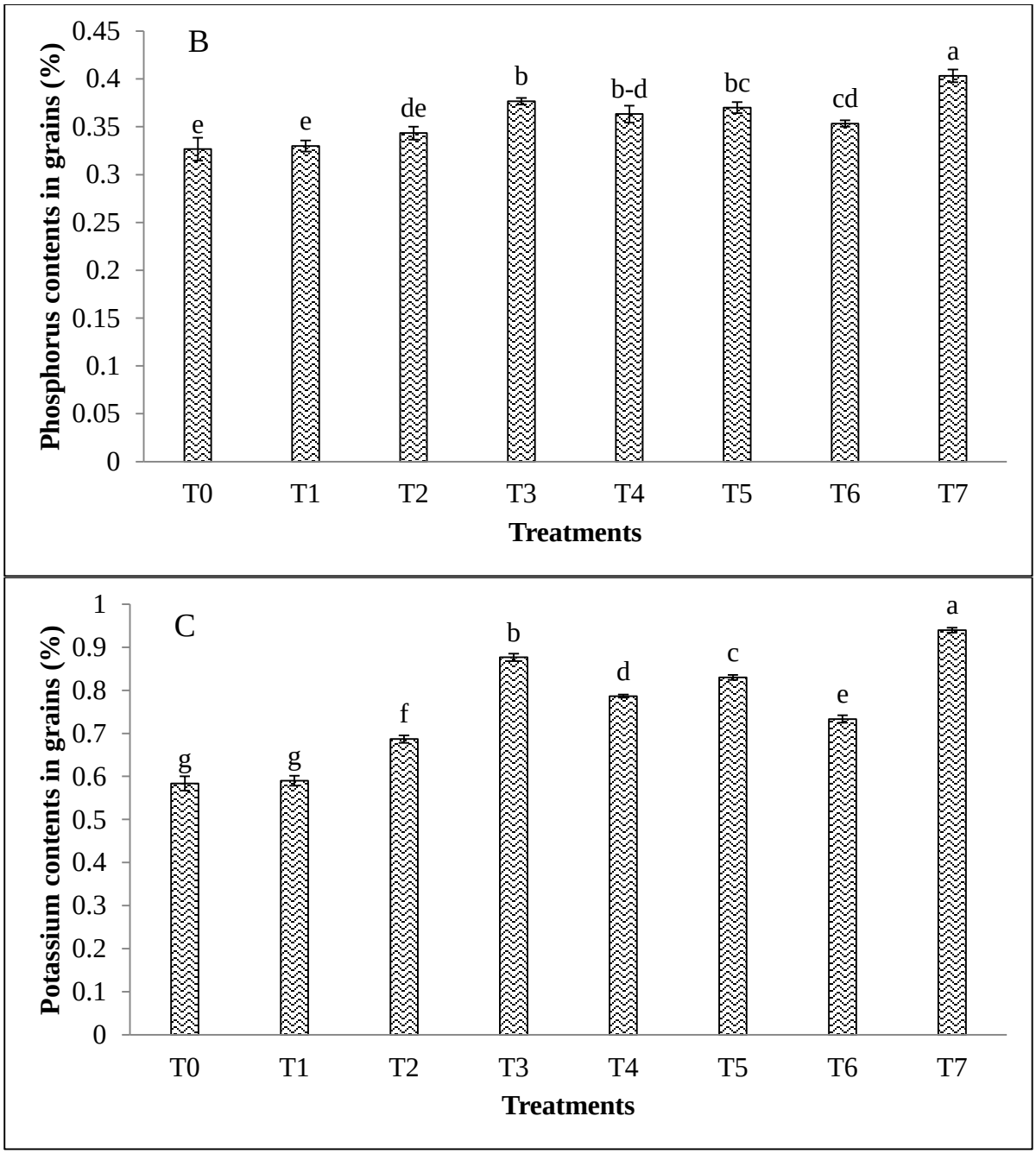


Figure-7. Effect of different dry region zinc solubilizing isolates coated on Zn coated urea on (A) nitrogen, (B) phosphorus, (C) potassium contents in grains (%) n = 3. Bars share similar letter(s) were not vary from each other at p≤0.05 statistically.

Zn and Fe in wheat grains

The results confirm that use of improved products (dry region Zn solubilizing bacteria coated on Zn coated urea) and already develop product (zabardast urea) along with $ZnSO_4$ significantly increase Zn and Fe in the grains of wheat (Figure 8 A and B). Improved product enhance the Zn and Fe up to 63% and 32%, respectively, however already develop product increase the Zn and Fe in grains up to 14.8 and 6.7% respectively, over control where no zinc source was used.

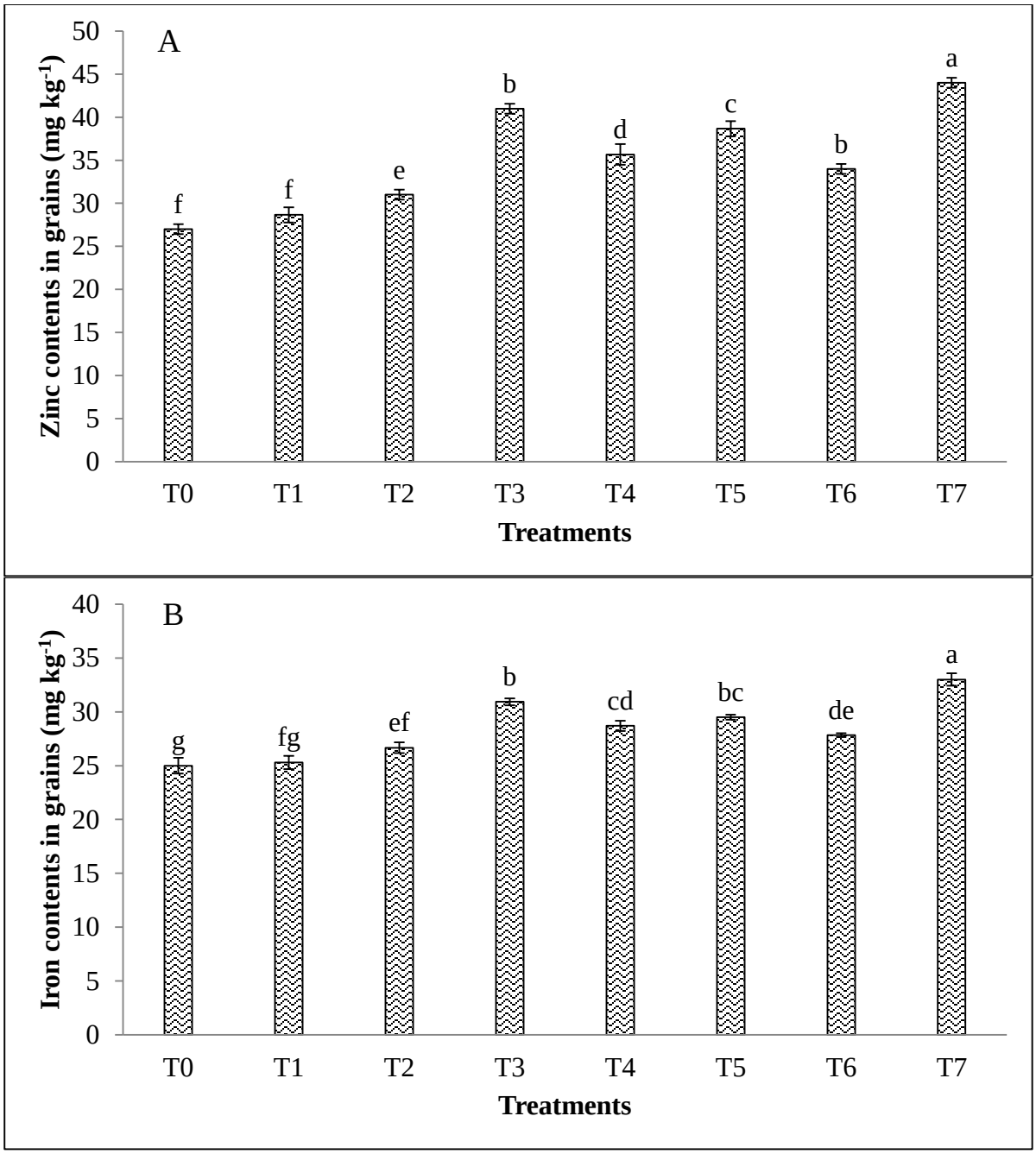


Figure-8. Effect of different dry region zinc solubilizing isolates coated on Zn coated urea on (A) Zn and (B) Fe contents in grains (mg kg-1) n = 3. Bars share similar letter(s) were not vary from each other at p≤0.05 statistically.

Discussion

In this study, pre-isolated dry region Zn solubilizing isolates was characterized for different plant growth improving characters e.g., siderophores production, ACC-deaminase and urease activity the results of these parameters confirm that rhizobacterial isolates possess plant growth promoting attributes. In this study, rhizobacterial strains showed the positive result for urease activity that were relate to the results of Mumtaz et al. (2017) which described our findings positive for the urease activity by *Bacillus* sp. strains. The urease activity changes the artificial or naturally occurring urea into carbonate and ammonia. Also, the

urease activity increases the amount of consumption of N containing fertilizers. In this way optimum amount of N becomes available for plants (Nosheen and Bano, 2014). Like our research, Mumtaz et al. (2017) described that production of siderophores capability in all identified *Bacillus* sp. isolates. Siderophores production is also an important process which plays role in the solubilization of Zn (Ajmal et al., 2021). Positive findings were shown from siderophore producing strain AZ6 Bacillus sp. documented by Hussain et al. (2015). It was noted that almost all rhizospheric isolates contain the capability for ACC-deaminase ability as evidence from the findings of ACC metabolism activity. When the strains were growing on substrate-ACC, all isolates perform growth but the cell density that examined was variable. These isolates possess a difference in the effectiveness to consume ACC as a sole source of nitrogen. As related to previous findings, the variation in the rate of utilization of ACC by rhizobacterial isolates have also described in prior research Nadeem et al. (2007) and Shaharoona et al. (2006) described that rhizobacterial strains consume ACC as a sole source of N but with difference in the degrees of effectiveness. Globally, especially in developing nations, the most common deficient micronutrient is Zn in human and plants because of crops were grown on such soils which have Zn deficiency (Zia et al., 2020). So, it is essential to solubilize the fixed or insoluble state of Zn because of the improvement of zinc bio-availability. The most efficient method to solubilize fixed form of Zn is the synthesis of organic acids (Javed et al., 2018; Mumtaz et al., 2019). Inoculation of these bacteria into soil was done where they synthesis organic acids, chelating agents and decrease Zn deficiency in soil (Masood et al., 2022), which ultimately fortify cereal gains, consumed as food (Krithika and Balachandar, 2016).

To solubilize the insoluble or fixed complexes of metal by organic acids production plays significant role in soil nutrient cycling (Rashid et al., 2016). Different researcher has examined that bacteria which solubilize Zn also synthesis different kinds of organic acids like gluconic acid, lactic acid and oxalic acid and ultimately decrease the soil pH and solubilize the insoluble Zn fraction in soil (Masood et al., 2022). Current research findings are related to the results of Prathap et al. (2022) and Bhakat et al. (2021), who found Zn solubilization potential in different bacterial strains. Earlier investigations reported that the presence of bacterial strains inoculated into broth amended with Zn3(PO4)2, ZnO and ZnCO3 resulted in the production of organic acids, which perform a significant role in the solubilization of Zn (Yadav et al., 2020). However, main organic acids synthesis by mostly Zn solubilizing isolates is the gluconic acid which play role in the insoluble minerals solubilization alongside various other organic acids. These findings similar with the explanations made by different researchers (Yadav et al., 2022). In present work, the selected isolates synthesis the acetic, malic, succinic, citric, gibberallic, gluconic and oxalic acid. Also, Javed et al. (2018) described that solubilization of Zn by rhizobacterial strains H-103, H-112 and H-84, synthesis the malic acid (up to 15.82, 8.18 and 8.92 µg mL^{-1} respectively), oxaloacetic acid (up to 2.49, 11.82 and 3.18 µg/mL respectively) and tartaric acid (up to 146.25, 90.78 and 147.05 µg mL^{-1} respectively). Zaheer et al. (2019) observe the synthesis of acetic acid, oxalic acid, malic acid, gluconic acid, citric acid, succinic acid and lactic acid in bacterial metabolites. Mumtaz et al. (2019) describes the synthesis of isobutyric acid, acetic acid, succinic acid, formic acid, isovaleric acid, citric acid lactic acid by *Bacillus subtilis* ZM63, *Bacillus cereus*, *Bacillus* sp. ZM20 in ZnO amended and control culture filtrate.

Pre-isolated dry region strain was used to characterize the plant growth promoting attributes and then coating on zinc coated urea fertilizer. Generally, root associated multifunction plant symbionts are bacilli commonly exist in rhizospheric soil (Singh et al., 2016, 2021a). They have enormous ability to colonize plant roots, host nourishment, and plant protection from abiotic and biotic stress conditions (Yadav et al., 2022; Singh et al., 2016, 2021a, b). The improved product of urea coated with Zn was applied to check the zinc temporal release and urease activity in the soil at growth room trial. It was observed that by the use of improved product of zinc coated urea, availability of Zn improved significantly (2.03 mg kg^{-1}) over control where no zinc was used and recommended dose of Zn at 75^{th} day of study. In soil, bio-activated zinc-coated urea releases slow and gradual zinc, for bio-activation of zinc (ZnO) use of organic material for zinc solubilizing bacteria acted like carrier material and chelation compounds for Zn (Shakeel et al., 2015). The combined use of PGPBs and Zn is the most efficient, easily adaptable and sustainable strategies that can successfully decrease human and plant Zn deficiency by increasing nutrition and wheat productivity (Jalal et al., 2022; Jalal et al., 2020; Jalal et al., 2023). Dry region zinc solubilizing isolates have

a great impact on the growth of wheat, physiology and yield under the natural environment. In this research work, a significant increase in wheat growth and development was seen as compared to control. Gandhi et al. (2023) revealed that bacterial inoculation in wheat seedlings increase the root length by 16-40%. Growth and yield improvement in wheat crop is due to diverse plant growth improving characters of Zn solubilizing isolates of dry region. Because growth of plant needs Zn, and when it exists in both higher and lower concentration, it can reduce the growth of plant due to toxicity and deficiency, respectively (Natasha et al., 2022). The research conducted by Eshaghi et al. (2019) and Zaheer et al. (2019) also documented the positive impact on plant development, growth and yield from both individual and co-inoculate the Zn solubilizing isolates. In the present study, coating of dry region zinc solubilizing isolates on zinc coated urea significantly improves the root length, plant height, root and shoot dry biomass of wheat over control. The results of present research work were related with Ahmad et al. (2019) who observed that inoculation of Zn solubilizing bacteria, *Bacillus subtilis* strain ZM63 and *Bacillus aryabhattai* strain S10 led to substantial improvements in root length, shoot length, shoot dry biomass and root dry biomass in maize crop. Additionally, Eshaghi et al. (2019) found the *Pseudomonas japonica* isolate F37 and F21 a led to notable improvements in maize shoot length, as well as dry and fresh weight, when compared to the control. The PGPR application in the wire-house study significantly enhanced growth and development of plant (Yadav et al., 2022; Singh et al., 2021b). These bacterial inoculants synthesis various growth hormones (Khan et al., 2020) and increase chlorophyll content (Mathivanan et al., 2017), initiation of physiological activities (Singh et al., 2021a,b; Meena et al., 2020), solubilization and mineralization of mineral elements (Kumari et al., 2016).

The present results confirm that application of dry region Zn solubilizing isolates improve the wheat productivity or yield because of these PGPBs play role in root system development and host plants biomass that act like an entrance for better absorption of nutrient for better performance of plant and higher production (Moretti et al., 2020). Earlier, the combination of Bacillus sp. inoculation with ZnO has been regarded as an efficient strategy to enhance various biochemical and physiological characteristics of maize, thereby boosting growth and yield of grains with elevated nutritional quality in field trials (Jalal et al., 2023). The simultaneous use of PGPBs and Zn enhanced Zn use effectiveness in tropical soils, leading to increased plant growth and yield in the wheat-maize cropping areas (Galindo et al., 2021). Earlier studies have indicated that the combine application of nano-Zn and PGPBs can effectively trigger the plant defense system by amplifying primary photosystems and metabolites, potentially resulting in increased plant growth and grains production (Tanveer et al., 2022).

The current experiment confirmed that Zn coated fertilizer coated with dry region zinc solubilizing isolates significantly enhance the N, P, K, Fe and Zn contents in wheat grains under pot conditions. This could be attributed to the PGPBs involvement in diverse soils and several mechanisms, including the synthesis of enzymes and phytohormones, biological nitrogen fixation and carboxylation, these mechanisms could aid in solubilization and accessibility of plants nutrients, facilitating enhanced absorption (Yasmin et al., 2022; Rehman et al., 2021). Particularly, studies have indicated that the inoculation of *Pseudomonas* sp. has the potential to enhance root architecture, proliferation and branching, this, in turn, can benefit host plant by enhancing their bio-chemical characteristics and increasing bioavailability of nutrient, thereby promoting plant health and resilience to adverse environmental situations (Yasmin et al., 2022; Abadi et al., 2021). Earlier research supports our findings, which demonstrating that inoculation with *P. fluorescens, B. subtilis* and *A. brasilense* can enhance nutrient absorption and promote the growth of sugarcane and various other cereal crops (Rosa et al., 2022; Galindo et al., 2022).

Conclusion

Coating of zinc solubilizing isolates of dry region on Zn coated urea is an effective approach for the biofortification of Zn and also minimizes the urea losses. Further this product was used for the biofortification of Zn in cereals and also used for plant growth improvement in dry areas. Because the dry region Zn solubilizing isolates have the capability to solubilize Zn in dry regions, due to heigh temperature, nutrients are not available for plants and urea volatilization process is also common. However, the results depicted that the use of dry region coated urea perform best to enhance Zn biofortification as compared to control (normal urea), but the results are

at par with Engro Zabardast Urea. So, it is concluded that this product (consortium coated on Zn coated urea) has the ability to increase the biofortification of zinc in dry areas and further this product application is recommended in field conditions to improve the quality of food crops.

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Contribution of Authors

Anwar H: The plan of experiment, preparation of material, data collection and analysis, tables and figures preparation, Prepared initial draft, Reviewed and finalized the draft.
Jamil M: Data validation, curation and editing, Reviewed and finalized the draft.
Hussain A: The plan of experiment, preparation of material, data collection and analysis, tables and figures preparation, Prepared initial draft, Data validation, curation and editing, Reviewed and finalized the draft.
Dar A: The plan of experiment, preparation of material, data collection and analysis, tables and figures preparation, Prepared initial draft, Reviewed and finalized the draft.
Ahmad M: The plan of experiment, preparation of material, data collection and analysis, tables and figures preparation, Reviewed and finalized the draft.
Salmen SH: Data validation, curation and editing, Reviewed and finalized the draft.
Ansari MJ: Data validation, curation and editing, Reviewed and finalized the draft.
Iqbal R: Prepared initial draft, Data validation, curation and editing, Reviewed and finalized the draft.

References

Abadi VAJM, Sepehri M, Khatabi B and Rezaei M, 2021. Alleviation of zinc deficiency in wheat inoculated with root endophytic fungus *Piriformospora indica* and Rhizobacterium *Pseudomonas putida*. Rhizosphere 17: 100311.

Ahmad M, Adil Z, Hussain A, Mumtaz MZ, Nafees M, Ahmad I and Jamil M, 2019. Potential of phosphate solubilizing Bacillus strains for improving growth and nutrient uptake in mungbean and maize crops. Pak. J. Agric. Sci. 56(2): 283-289.

Ahmad S, Mfarrej MFB, El-Esawi MA, Waseem M, Alatawi A, Nafees M, Saleem MH, Rizwan M, Yasmeen T, Anayat A and Ali S, 2022. Chromium-resistant Staphylococcus aureus alleviates chromium toxicity by developing synergistic relationships with zinc oxide nanoparticles in wheat. Ecotoxicol. Environ. Saf. 230: 113142.

Ajmal AW, Saroosh S, Mulk S, Hassan MN, Yasmin H, Jabeen Z, Nosheen A, Shah SMU, Naz R, Hasnain Z and Qureshi TM, 2021. Bacteria isolated from wastewater irrigated agricultural soils adapt to heavy metal toxicity while maintaining their plant growth promoting traits. Sustainability 13(14): 7792.

Alef K and Nannipieri P, 1995. Methods in applied soil microbiology and biochemistry. Academic Press, San Diego, USA.

Alexander M, 1983. Most probable number method for microbial populations. In: A.L. Page, R.H. Miller, D.R. Keeney (Eds), Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties, 2nd Edn. Agronomy Monograph No. 9. American Society of Agronomy, Inc., and Soil Science Society of America, Inc. Madison, WI. pp. 815–820.

Bhakat K, Chakraborty A and Islam E, 2021. Characterization of zinc solubilization potential of arsenic tolerant Burkholderia spp. isolated from rice rhizospheric soil. World J. Microbiol. Biotechnol. 37: 1-13.

Bouis HE and Welch RM, 2010. Biofortification a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Sci. 50: S-20.

Broadley M, Brown P, Cakmak I, Rengel Z and Zhao F, 2012. Function of nutrients: micronutrients. In Marschner's mineral nutrition of higher plants.

Academic Press. pp. 191-248.
Bunt JS and Rovira AD, 1955. Microbiological studies of some subantarctic soils. J. Soil Sci. 6(1): 119-128.
Butsat S, Weerapreeyakul N and Siriamornpun S, 2009. Changes in phenolic acids and antioxidant activity in Thai rice husk at five growth stages during grain development. J. Agric. Food Chem. 57(11): 4566-4571.
Cakmak I, 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification?. Plant Soil. 302: 1-17.
Cappuccino JC and Sherman N, 2005. Microbiology: A laboratory manual. Benjamin/ Cummings Publishing Company, New York. pp. 125-179.
De Valença AW, Bake A, Brouwer ID and Giller KE, 2017. Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. Glob. Food Secur. 12: 8-14.
Dworkin M and Foster J, 1958. Experiments with some microorganisms which utilize ethane and hydrogen. J. Bacteriol. 75(5): 592-603.
Eshaghi E, Nosrati R, Owlia P, Malboobi MA, Ghaseminejad P and Ganjali MR, 2019. Zinc solubilization characteristics of efficient siderophore-producing soil bacteria. Iran. J. Microbiol. 11(5): 419.
Fasim F, Ahmed N, Parsons R and Gadd GM, 2002. Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery. FEMS Microbiol. Lett. 213(1): 1-6.
Fukui R, Schroth MN, Hendson M and Hancock JG, 1994. Interaction between strains of pseudomonads in sugar beet spermospheres and their relationship to pericarp colonization by *Pythium ultimum* in soil. Phytopathol. 84(11): 1322-1330.
Galindo FS, Bellotte JLM, Santini JMK, Buzetti S, Rosa PAL, Jalal A and Teixeira Filho MCM, 2021. Zinc use efficiency of maize-wheat cropping after inoculation with Azospirillum brasilense. Nutr. Cycling Agroecosyst. 120(2): 205-221.
Galindo FS, Rodrigues WL, Fernandes GC, Boleta EHM, Jalal A, Rosa PAL, Buzetti S, Lavres J and Teixeira Filho MCM, 2022. Enhancing agronomic efficiency and maize grain yield with Azospirillum brasilense inoculation under Brazilian savannah conditions. Eur. J. Agron. 134: 126471.
Gandhi R, Prittesh, P, Jinal HN, Chavan SM, Paul D and Amaresan N, 2023. Evaluation of the effect of potassium solubilizing bacterial strains on the growth of wheat (*Triticum aestivum* L.). J. Plant Nutr. 46(8): 1479-1490.
Gopalakrishnan S, Pande S, Sharma M, Humayun P, Kiran BK, Sandeep D, Vidya MS, Deepthi K and Rupela O, 2011. Evaluation of actinomycete isolates obtained from herbal vermicompost for the biological control of Fusarium wilt of chickpea. Crop Prot. 30(8): 1070-1078.
Gurmani AR, Khan SU, Andaleep R, Waseem K and Khan A, 2012. Soil Application of zinc improves growth and yield of tomato. Int. J. Agric. Biol. 14(1): 91-96.
Haroon M, Khan ST and Malik A, 2022. Zinc-Solubilizing Bacteria: An Option to Increase Zinc Uptake by Plants. In: Khan ST, Malik A (Eds) Microbial Biofertilizers and Micronutrient Availability. Springer, Cham. pp 207-238.
Hashemnejad F, Barin M, Khezri M, Ghoosta Y and Hammer EC, 2021. Isolation and identification of insoluble zinc-solubilising bacteria and evaluation of their ability to solubilise various zinc minerals. J. Soil Sci. Plant Nutr. 21: 2501-2509.
Huang S, Yamaji N and Feng Ma J, 2022. Zinc transport in rice: how to balance optimal plant requirements and human nutrition. J. Exp. Bot. 73(6): 1800-1808.
Hussain A, Arshad M, Zahir ZA and Asghar M, 2015. Prospects of zinc solubilizing bacteria for enhancing growth of maize. Pak. J. Agric. Sci. 52(4): 915-922.
Imran M, Arshad M, Khalid A, Kanwal S and Crowley DE, 2014. Perspectives of rhizosphere microflora for improving Zn bioavailability and acquisition by higher plants. Int. J. Agric. Biol. 16(3): 653-662.
Jackson ML, 1962. Soil Chemical Analysis. Prentice Hall, Inc., Englwood Cliff, New York, USA.
Jalal A, da Silva Oliveira CE, Freitas LA, Galindo FS, Lima BH, Boleta EHM, Da Silva EC, do Nascimento V, Nogueira TAR, Buzetti S and Teixeira Filho MCM, 2022. Agronomic biofortification and productivity of wheat with soil zinc and diazotrophic bacteria in tropical savannah. Crop Pasture Sci. 73: 817-830.
Jalal A, Oliveira CEDS, Bastos ADC, Fernandes GC, De Lima BH, Furlani Junior E, De Carvalho PHG, Galindo FS, Gato IMB and Teixeira Filho MCM, 2023. Nanozinc and plant growth-promoting

bacteria improve biochemical and metabolic attributes of maize in tropical Cerrado. Front. Plant Sci. 13: 1046642.

Jalal A, Shah S, Carvalho Minhoto Teixeira Filho M, Khan A, Shah T, Ilyas M and Leonel Rosa PA, 2020. Agronomische Biofortifikation von Zink und Eisen in Weizenkörnern. Gesunde Pflanzen. 72: 227-236.

Javed H, Akhtar MJ, Asghar HN and Jamil A, 2018. Screening of zinc solubilizing bacteria and their potential to increase grain concentration in wheat (*Triticum aestivum* L). Int. J. Agric. Biol. 20(3): 547-553.

Jha Y, 2019. The importance of zinc-mobilizing rhizosphere bacteria to the enhancement of physiology and growth parameters for paddy under salt-stress conditions. Jordan J. Biol. Sci. 12(2): 167-173.

Khan N, Bano A, Ali S and Babar MA, 2020. Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. Plant Growth Regul. 90: 189-203.

Krithika S and Balachandar D, 2016. Expression of zinc transporter genes in rice as influenced by zinc-solubilizing Enterobacter cloacae strain ZSB14. Front. Plant Sci. 7: 183661.

Kumari P, Sharma B, Kumari R and Murya BR, 2016. Soil microbial dynamics as influenced by organic amendments in alluvium soil of indo-gangetic plains. India. J. Pure Applied Microbiol. 10: 2919–2924.

Mahuku GS, 2004. A simple extraction method suitable for PCR-based analysis of plant, fungal, and bacterial DNA. Plant Mol. Biol. Rep. 22: 71-81.

Masood F, Ahmad S and Malik A, 2022. Role of Rhizobacterial Bacilli in Zinc Solubilization. In: Khan, ST, Malik, A (Eds) Microbial Biofertilizers and Micronutrient Availability. Springer, Cham. pp 361–377.

Mathivanan S, Chidambaram ALA, Robert GA and Kalaikandhan R, 2017. Impact of PGPR inoculation on photosynthetic pigment and protein. J. Sci. Agric. 1: 29-36.

Mayer JE, 2008. Biofortification of crops to alleviate micronutrient malnutrition. Plant Biol. 11: 1-15.

Meena M, Swapnil P, Divyanshu K, Kumar S, Harish Tripathi YN, Zehra A, Marwal A and Upadhyay RS, 2020. PGPR-mediated induction of systemic resistance and physiochemical alterations in plants against the pathogens: Current perspectives. J. Basic Microbiol. 60(10): 828-861.

Mirza FM, Najam N, Mehdi M and Ahmad B, 2015. Determinants of technical efficiency of wheat farms in Pakistan. Pak. J. Agric. Sci. 52(2): 565-570.

Mohammadi H, Talebi S, Ghavami A, Rafiei M, Sharifi S, Faghihimani Z, Ranjbar G, Miraghajani M and Askari G, 2021. Effects of zinc supplementation on inflammatory biomarkers and oxidative stress in adults: A systematic review and meta-analysis of randomized controlled trials. J. Trace Elem. Med. Biol. 68: 126857.

Moodie CD, Smith W and MeCreery RA, 1959. Laboratory Manual for soil fertility. pp. 1–75. Dept. Agron, State College of Washington. Pullman, Washington.

Moretti LG, Crusciol CA, Kuramae EE, Bossolani JW, Moreira A, Costa NR, Alves CJ, Pascoaloto IM, Rondina AB and Hungria M, 2020. Effects of growth-promoting bacteria on soybean root activity, plant development, and yield. Agron. J. 112(1): 418-428.

Mumtaz MZ, Ahmad M, Jamil M and Hussain T, 2017. Zinc solubilizing Bacillus spp. potential candidates for biofortification in maize. Microbiol. Res. 202: 51-60.

Mumtaz MZ, Barry KM, Baker AL, Nichols DS, Ahmad M, Zahir ZA and Britz ML, 2019. Production of lactic and acetic acids by Bacillus sp. ZM20 and Bacillus cereus following exposure to zinc oxide: A possible mechanism for Zn solubilization. Rhizosphere. 12: 100170.

Nadeem SM, Zahir ZA, Naveed M and Arshad M, 2007. Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Can. J. Microbiol. 53(10): 1141-1149.

Natasha N, Shahid M, Bibi I, Iqbal J, Khalid S, Murtaza B, Bakhat HF, Farooq ABU, Amjad M, Hammad HM and Niazi NK, 2022. Zinc in soil-plant-human system: A data-analysis review. Sci. Total Environ. 808: 152024.

Naz I, Ahmad H, Khokhar SN, Khan K and Shah AH, 2016. Impact of zinc solubilizing bacteria on zinc contents of wheat. Am. Euras. J. Agric. Environ. Sci. 16: 449-454.

Nazir Q, Hussain A, Imran M, Mahmood S, Ahmad M and Iqbal MM, 2016. Zinc biofortification of cereals through fertilizers: Recent advances and future perspectives. Asian J. Agric. Biol. 4: 40-152.

Nosheen A and Bano A, 2014. Potential of plant growth promoting rhizobacteria and chemical fertilizers on soil enzymes and plant growth. Pak. J. Bot. 46(4): 1521-1530.

Peck AW and McDonald GK, 2010. Adequate zinc nutrition alleviates the adverse effects of heat stress in bread wheat. Plant and soil. 337: 355-374.

Penrose DM and Glick BR, 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol. Plant. 118(1): 10-15.

Perrière G and Gouy M, 1996. WWW-query: an on-line retrieval system for biological sequence banks. Biochimie. 78(5): 364-369.

Potarzycki J and Grzebisz W, 2009. Effect of zinc foliar application on grain yield of maize and its yielding compone. Plant Soil Environ. 55(12): 519-527.

Prathap S, Thiyageshwari S, Krishnamoorthy R, Prabhaharan J, Vimalan B, Gopal NO and Anandham R, 2022. Role of zinc solubilizing bacteria in enhancing growth and nutrient accumulation in rice plants (*Oryza sativa*) grown on zinc (Zn) deficient submerged soil. J. Soil Sci. Plant Nut. 22: 971-984.

Rashid A and Ryan J, 2008. Micronutrient constraints to crop production in the Near East: Potential significance and management strategies. In: Alloway BJ (Eds) Micronutrient Deficiencies in Global Crop Production. Springer, Dordrecht. pp. 149-180.

Rashid MI, Mujawar LH, Shahzad T, Almeelbi T, Ismail IM and Oves M, 2016. Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. Microbiol. Res. 183: 26-41.

Rehman A, Farooq M, Ozturk L, Asif M and Siddique KH, 2018. Zinc nutrition in wheat-based cropping systems. Plant Soil. 422: 283-315.

Rehman R, Asif M, Cakmak I and Ozturk L, 2021. Differences in uptake and translocation of foliar-applied Zn in maize and wheat. Plant Soil. 462: 235-244.

Rosa PAL, Galindo FS, Oliveira CEDS, Jalal A, Mortinho ES, Fernandes GC, Marega EMR, Buzetti S and Teixeira Filho MCM, 2022. Inoculation with plant growth-promoting bacteria to reduce phosphate fertilization requirement and enhance technological quality and yield of sugarcane. Microorganisms. 10(1): 192.

Ryan J, Estefan G and Rashid A, 2001. Soil and plant analysis laboratory manual. ICARDA.

Samaras A, Kamou N, Tzelepis G, Karamanoli K, Menkissoglu-Spiroudi U and Karaoglanidis GS, 2022. Root transcriptional and metabolic dynamics induced by the plant growth promoting rhizobacterium (PGPR) Bacillus subtilis Mbi600 on cucumber plants. Plants. 11(9): 1218.

Saravanan VS, Madhaiyan M and Thangaraju M, 2007. Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium Gluconacetobacter diazotrophicus. Chemosphere. 66(9): 1794-1798.

Saravanan VS, Subramoniam SR and Raj SA, 2004. Assessing in vitro solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. Braz. J. Microbiol. 35: 121-125.

Sbartai H, Djebar MR, Rouabhi R, Sbartai I and Berrebbah H, 2011. Antioxidative response in tomato plants Lycopersicon esculentum L. roots and leaves to zinc. Am. Eurasian. J. Toxicol. Sci. 3(1): 41-46.

Shaharoona B, Arshad M and Zahir ZA, 2006. Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). Lett. Appl. Microbiol. 42(2): 155-159.

Shaikh SS and Saraf MS, 2017. Optimization of growth conditions for zinc solubilizing plant growth associated bacteria and fungi. J. Adv. Res. Biotechnol. 2(1): 1-9.

Shakeel M, Rais A, Hassan MN and Hafeez FY, 2015. Root associated Bacillus sp. improves growth, yield and zinc translocation for basmati rice (Oryza sativa) varieties. Front. Microbiol. 6: 166077.

Singh S, Singh UB, Trivdi M, Malviy D, Sahu PK, Roy M, Sharma PK, Singh HV, Manna MC and Saxena AK, 2021a. Restructuring the cellular responses: connecting microbial intervention with ecological fitness and adaptiveness to the maize (*Zea mays* L.) grown in saline–sodic soil. Front. Microbiol. 11: 568325.

Singh UB, Malviya D, Singh S, Pradhan JK, Singh BP, Roy M, Imram M, Pathak N, Baisyal, BM, Rai JP and Sarma BK, 2016. Bio-protective microbial agents from rhizosphere eco-systems trigger plant defense responses provide protection against sheath blight disease in rice (Oryza sativa L.). Microbiol. Res. 192: 300-312.

Singh UB, Malviya D, Singh S, Singh P, Ghatak A, Imran M, Rai JP, Singh RK, Manna MC, Sharma AK and Saxena AK, 2021b. Salt-tolerant compatible microbial inoculants modulate physio-biochemical responses enhance plant growth, Zn biofortification and yield of wheat grown in saline-sodic soil. Int. J. Environ. Res. Public Health. 18(18): 9936.

Soltanpour PN and Schwab AP, 1977. A new soil test for simultaneous extraction of macro-and micro-nutrients in alkaline soils. Commun. Soil Sci. Plant Anal. 8(3): 195-207.

Soltanpour PN and Workman S, 1979. Modification of the NH4 HCO3-DTPA soil test to omit carbon black. Commun. Soil Sci. Plant Anal. 10(11): 1411-1420.

Steel R, Torrie J and Dieky D, 1997. Principles and procedures of statistics, 3rd Edn. New York, NY: McGraw Hill Book Co Inc.

Tanveer Y, Yasmin H, Nosheen A, Ali S and Ahmad A, 2022. Ameliorative effects of plant growth promoting bacteria, zinc oxide nanoparticles and oxalic acid on Luffa acutangula grown on arsenic enriched soil. Environ. Pollut. 300: 118889.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F and Higgins DG, 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25(24): 4876-4882.

U.S. Salinity Laboratory Staff, 1954. Diagnosis and improvement of saline and alkali soils. USDA Hand Book No. 60. Washington, DC, USA.

United Nations Development Programme (UNDP), 2003. Human development report. 2003. The millennium development goals: a compact among nations to end human poverty. New York, USA.

Walkley A and Black IA, 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37(1): 29-38.

Waqeel J and Khan ST, 2022. Microbial Biofertilizers and Micronutrients Bioavailability: Approaches to Deal with Zinc Deficiencies. In: Khan ST and Malik A (Eds) Microbial Biofertilizers and Micronutrient Availability. Springer, Cham. pp. 239-297.

Watanabe FS and Olsen SR, 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Am. J. 29(6): 677-678.

WHO, 2012. World Health Report 2012: Reducing Risks, Promoting Healthy Life. World Health Organization, Geneva, Switzerland.

Yadav RC, Sharma SK, Ramesh A, Sharma K, Sharma PK and Varma A, 2020. Contribution of Zinc-Solubilizing and-Mobilizing Microorganisms (ZSMM) to enhance zinc bioavailability for better soil, plant, and human health. In Sharma, S.K., Singh, U.B., Sahu, P.K., Singh, H.V., Sharma, P.K. (eds) Rhizosphere Microbes: Microorganisms for Sustainability, vol 23. Springer, Singapore. pp 357-386.

Yadav RC, Sharma SK, Varma A, Rajawat MVS, Khan MS, Sharma PK, Malviya D, Singh UB, Rai JP and Saxena AK, 2022. Modulation in biofertilization and biofortification of wheat crop by inoculation of zinc-solubilizing rhizobacteria. Front. Plant Sci. 13: 777771.

Yaqoob N, Jain V, Atiq Z, Sharma P, Ramos-Meza CS, Shabbir MS and Tabash MI, 2022. The relationship between staple food crops consumption and its impact on total factor productivity: does green economy matter? Environ. Sci. Pollut. Control Ser. 1–10.

Yasmin H, Bano A, Wilson NL, Nosheen A, Naz R, Hassan MN, Ilyas N, Saleem MH, Noureldeen A, Ahmad P and Kennedy I, 2022. Drought-tolerant Pseudomonas sp. showed differential expression of stress-responsive genes and induced drought tolerance in Arabidopsis thaliana. Physiol. Plant. 174(1): e13497.

Zaheer A, Malik A, Sher A, Qaisrani MM, Mehmood A, Khan SU, Ashraf M, Mirza Z, Karim S and Rasool M, 2019. Isolation, characterization, and effect of phosphate-zinc-solubilizing bacterial strains on chickpea (Cicer arietinum L.) growth. Saudi J. Biol. Sci. 26(5): 1061-1067.

Zia MH, Ahmed I, Bailey EH, Lark RM, Young SD and Lowe NM, 2020. Site-specific factors influence the field performance of a Zn-biofortified wheat variety. Front. Sust. Food Syst. 4: e135. DOI: https://doi.org/10.3389/fsufs.2020.00135.

Zou CQ, Zhang YQ, Rashid A, Ram H, Savasli E, Arisoy RZ, Ortiz-Monasterio I, Simunji S, Wang ZH, Sohu V and Hassan MU, 2012. Biofortification of wheat with zinc through zinc fertilization in seven countries. Plant Soil. 361: 119-130.