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Investigating the Efficacy of Dry Region Zinc Solubilizing Bacteria for Growth Promotion of Maize and Wheat under Axenic Conditions

[a]Muhammad Umair Asghar, [a]Azhar Hussain*, [a]Hammad Anwar, [a]Abubakar Dar, [b]Hafiz Tanvir Ahmad, [c]Qudsia Nazir, [d]Nadeem Tariq, [e]Muhammad Usman Jamshaid

[a] Department of Soil Science, The Islamia University of Bahawalpur, 63100-Pakistan.
[b] National Cotton Breeding Institute, The Islamia University of Bahawalpur, 63100-Pakistan.
[c] Institute of Soil Chemistry and Environmental Sciences, Ayub Agriculture Research Institute, Faisalabad, Pakistan.
[d] Microbial Biotechnologies (Private) Limited Lahore, Pakistan.
[e] Department of Soil and Environmental Science, Muhammad Nawaz Sharif University of Agriculture Multan, Pakistan.

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ABSTRACT

Zinc (Zn) plays a crucial role as a micronutrient, essential for the growth, development, and proper functioning of plants. Pakistani soils are deficient of micronutrient especially zinc. Supplementation of Zn fertilizers cannot do the job because only a small fraction of Zn is available to plants and rest is fixed in soil. To enhance the Zn availability different methods are being used which are expensive. Zinc solubilizing bacteria (ZSB) can be a potential candidate for solubilization of Zn and growth promoting activities in cereals. The present study aimed to evaluate the potential of dry region bacterial strains of Bahawalpur for improving Zn uptake and growth improvement in wheat and maize. Rhizosphere samples of wheat and maize were taken from the dry regions of Bahawalpur and bacterial strains were isolated and tested for their Zn solubilizing ability and screened for urease, siderophore activity, exopolysaccharide production, phosphorus solubilization and plant growth promoting abilities. Under axenic conditions selected isolates were further screened to assessed for improving the growth of wheat and maize seedlings. The rhizobacterial isolates IUB-34, IUB-80, IUB-93 and IUB-96 performed best to improve growth, physiology, biochemical attributes and root colonization. The maximum increase in root and shoot lengths were recorded 32.2 and 35.7% in wheat under IUB-34 application and in maize 43.8 and 39.9%, respectively, under IUB-96 application as compared to uninoculated control. Furthermore, maximum root colonization was also recorded under IUB-34 in wheat and IUB-96 in maize seedlings. Moreover, the biochemical characterization of selected isolates showed IAA (Auxins) production, protease, catalase, cellulose degradation, HCN and ACC-deaminase activities by these strains. However, only IUB-34 and IUB-96 were found positive for oxidase activity. Therefore, study concluded that use of dry region ZSB can solubilize Zn and make it available for plant to improve growth of wheat and maize. So, these isolates may be utilized for coating urea as a biofertilizer to increase the biofortification in cereals.

Corresponding Author: Azhar Hussain
Email: azharhaseen@gmail.com

INTRODUCTION

Despite rapid progress in industrial and technological sectors, agriculture continues to be Pakistan's most vital industry, contributing one-fifth of the nation's total gross domestic product (GDP) (Mirza *et al*. 2015). Wheat is a staple crop of Pakistan's people as the majority is consuming it three times daily in the form of chapati and pancakes (Ahsin *et al*. 2020). Maize (*Zea mays* L.) holds significant importance as a versatile cereal crop within the *Poaceae* family. In Pakistan, it ranks as the third most vital cereal grain following wheat and rice. It serves as food for humans, a raw material for industries, and a feed for livestock (Kaushal *et al*. 2023). Despite the diverse potential of various maize cultivars, the average production remains comparatively low due to several management constraints and agronomic practices that haven't fully tapped into its production potential (Sarwar and Biswas 2021). One of the primary constraints is related to plant nutrition, with Zn playing a pivotal role for the growth of plants, humans, and animals. However, Zn deficiency persists in our soils, leading to various physiological and developmental challenges in plants, hindering their normal functioning (Sahoo *et al*. 2021; Ullah *et al*. 2023).

Farming community has shown their increasing interest in adopting innovative approaches to tackle productivity and address micronutrient deficiencies, commonly known as hidden hunger in most cereal grains, a food source for over 2 billion individuals globally (Waqeel and Khan 2022). About 40% children and women in Pakistan are affected by the Zn deficiency (GOP, 2009). Globally Zn deficiency cause 10% diarrhea, 16% respiratory disorder and 18% malaria, with annually 0.8 million deaths (WHO, 2012). Zinc is essential for the healthy growth and development of plants, contributing to carbohydrate metabolism and plant auxin regulation (Hussain *et al*. 2019). Additionally, Zn serves as an activator of antioxidant defense system in plants and its deficiency results in smaller leaves, slower development and chlorosis (Zafar *et al*. 2018). Zinc deficiency is a common concern in Pakistan, where calcareous soils are widely utilized for cultivating staple crops (Younas *et al*. 2023). Food and Agriculture Organization (FAO) reported that approximately half of the agricultural soils of the world suffers from Zn deficiency. The scenario in Pakistan is even worst as seventy percent Pakistani soil showed zinc deficiency (Imtiaz *et al*. 2010). In Pakistani soils Zn deficiency are approximately 70% due to calcareous and alkaline nature, high pH, low organic matter, high temperature and calcium carbonate in soil (Hussain *et al*., 2018; Imran *et al*., 2014). Moreover, the application of Zn containing fertilizers (Zn-EDTA or Zinc sulfate) often provide an ineffective availability of Zn due to precipitation with phosphates, carbonates and oxides (Zhang *et al*. 2017). Different alternative strategies, including genetic engineering, traditional breeding, transgenic crops have been explored to deal with Zn deficiency, yet these methods are costly, time-intensive, and relatively slow (Wang *et al*. 2020; Khokhar *et al*. 2020).

Climate change has a significant impact on the global agriculture system (Wiebe *et al*. 2019; Ye *et al*. 2019). Another constrain in cereal production is the water scarcity which is becoming major challenge for agricultural production in the near future as an estimate depicted acute water scarcity in Pakistan by 2025 (Khan *et al*. 2022). Water scarcity is closely linked with the nutrient (especially micronutrients) availability to plants by precipitating them in the soil (Havrlentová *et al*. 2021; Anwar Ul Haq *et al*. 2023). So, the cereals production in dry regions of Pakistan will also subject water scarcity and reduced availability of nutrients in cereals. The immigrate need of the hour is to use such technologies to cope not only with malnutrition but also water associated nutrient deficiency in cereals in economical, ecofriendly and time saving manner. Plant growth-promoting rhizobacteria (PGPR) have been studied as drought tolerant and efficient Zn solubilizers by adopting different mechanisms including exopolysaccharides (EPS) production and Zn conversion into more available forms (Hussain *et al*. 2019; Singh and Prasanna 2020).

Microorganisms employ diverse mechanisms to solubilize Zn, including acidification through the production of organic acids, which lowers the pH and sequesters Zn cations (Rasul *et al*. 2019). Siderophore production being associated in Zn solubilization by chelating Zn and making it available to plants (Ajmal *et al*. 2021). Moreover, the EPS production by bacteria can alleviate drought stress by holding more water in the soil and nutrients solubilization in cereals (Latif *et al*. 2022). Multiple PGPRs have demonstrated enhanced crop growth and Zn contents in plants, with notable strains including *Bacillus aryabhattai, Rhizobium, Bacillus subtilis* and *Pseudomonas*, (Prathap *et al*. 2022; Samaras *et al*. 2022).

Moreover, their ability to solubilize insoluble Zn ($ZnCO_3$ and ZnO) is already studied with reference to other nutrients solubilization through the production of organic acids siderophores and EPS production (Etesami and Adl 2020; Santoyo *et al*. 2021). Plant growth promoting rhizobacteria produce siderophores

(Saravanan *et al*. 2011), gluconic acid which are the derivatives of e.g., 5-ketogluconic acid (Saravanan *et al*. 2007), 2- ketogluconic acid (Fasim *et al*., 2002) and various organic acids for Fe and Zn mobilization (Tariq *et al*., 2007). Rhizobacteria also release vitamins, amino acids, and chelating ligands that have the capability to solubilize insoluble Zn compounds and make them available (Saravanan *et al*. 2004). Different Zn solubilizing rhizobacterial species like *Gluconacetobacter* (Intorne *et al*. 2009), *Pseodomonas* (Bapiri *et al*. 2012), *Burkholderia* and *Serratia* (Dinesh *et al*. 2015), *Bacillus* (Mumtaz *et al*. 2017 and 2018) are described. In addition to Zn solubilization, these strains have the capability to nitrogen fixation (El-Sayed *et al*. 2014), phosphorus solubilization, production of different kind of enzymes like oxidase, catalase, lipase, protease, chitinase and also produce exopolysaccharides and phytohormones (Mumtaz *et al*., 2017; Imran *et al*., 2014). Antagonistic ability of these bacteria also reported against pathogenic fungi such as *Fusarium oxysporum*, *Sclerotinia clerotiorum* and some nematodes species like Meloidogyne incognita (El-Sayed *et al*. 2014).

Considering the consequences of climate change Zn malnutrition and the role of soil microorganisms to cope with these problems, the present study was designed on specific objective to isolate, screen and investigate the potential of Zn-solubilizing bacteria (ZSB) of dry region of Bahawalpur in alleviating Zn malnutrition, enhancing crop growth and Zn accumulation in wheat and maize under axenic conditions. It was hypothesized that the application of dry region ZSB strains may enhance the growth and Zn contents in wheat and maize under axenic conditions.

MATERIALS AND METHODS

Isolation of Rhizobacterial Isolates from Maize and Wheat Rhizosphere Soil

Isolation from rhizosphere soil of wheat and maize crop was done from dry region of Bahawalpur including Yazman, Rahim Yar khan, Fort Abbas and research area Department of Soil Science, The Islamia University of Bahawalpur. Wheat and maize plants were uprooted with earth balls and soil adhering with roots were collected. The isolation of the dry region rhizobacteria was done through serial dilution method by diluting the sample up to 10^8 on DF minimal media amended with 0.1 % ZnO per liter as described by Wollum (1983) and incubated for 48 hours at 30 ± 1 °C in an incubator. Afterward, the isolated colonies were re-streaked on new DF minimal agar plates and repeated 3-4 times to get purified colonies. After purification 60 bacterial strains of pure culture each from wheat and maize were preserved in 50% glycerol at -30 °C for further analysis.

In-vitro Screening of the Dry Region Rhizobacterial Isolates for Plant Growth Promoting Attributes

The rhizobacterial isolates were examined for plant growth promoting characteristics in term of Zn solubilization, urease activity, siderophores, exopolysaccharides production and phosphorus solubilization. Bacterial isolates were grown on DF minimal media for screening of different characteristics (Dworkin and Foster 1958). The fresh culture of bacterial isolates for Zn solubilization were inoculated on tris-minimal salt media amended with 0.1% zinc oxide (Fasim *et al*. 2002) and incubate at 30 ± 1 °C for 72 hrs for determination of clearing zone around colonies. Colony and holo zone diameter were recorded to calculate the solubilization efficiency and solubilization index of Zn by using formula described by Ahmad *et al*. (2019a). For quantitative Zn solubilization bacterial isolates were inoculated on tris-minimal media amended with 0.1% zinc oxide and incubated at 30 ± 1 °C in shaking incubator for 72 h. After incubation period the bacterial cells were harvested by centrifugation and the supernatant was analyzed for Zn on atomic absorption spectrophotometer by comparing with known standards.

Christensen's urea broth was inoculated with bacterial isolates and incubated at 30 ± 1 °C for 24 hours. The change in color from the pale orange to pink confirms the urease activity of bacterial isolates (Cappuccino and Sherman, 2002). For phosphorus determination bacterial isolates were spot inoculated on Pikovskaya's agar media and incubate at 30 ± 1 °C for 72 h (Pikovskaya 1948). Siderophores production of isolates was done on chrome azurol S agar media and incubated for 48 h at 30 ± 1 °C (Schwyn and Neilands 1987). Appearance of the orange color zone around microbial colonies confirm the positive siderophore production. For exopolysaccharide production bacterial isolates were streaked on RCV glucose agar media and incubated for 48 h at 30 ± 1ºC. Mucoidal growth around bscterial colonies confirms the exopolysaccharide production (Strieth *et al*. 2021).

Effect of Zn Solubilizing Isolates on Maize and Wheat

Growth Under Axenic Condition

The growth promoting ability of prominent Zn solubilizing bacterial isolates of wheat and maize of dry region were further confirmed on wheat and maize seedlings under axenic condition. Inoculum of ten (each from wheat and maize) isolates of Zn solubilizing dry region bacteria was prepared in LB broth media. Surface disinfection of wheat and maize seeds was done by dipping seeds of both cereals in mercuric chloride (0.2%) for 1 minutes and ethanol (95%) for 30 s followed by rinsing by sterilized distilled water 5-6 times as described by Al-Adham *et al*. (2012). Seed of both cereals were primed with their respective ZSB of dry climate by dipping in broth culture for 20 min prior to sowing. Plastic jars (11cm height, 11cm width) were filled with 600 g sand and moisturized with half-strength Hoagland solution and autoclaved. After cooling 10 seeds were sown in each pot for wheat and 5 seeds per pot for maize and placed in growth room following completely randomize design replicated thrice. Conditions for wheat are 12 h dark at 15 ± 2 °C and 12 h light at 25 ± 2 °C with 70-90% relative humidity and for maize crop grown in growth room at 14-28 °C with 60-75% relative humidity. After germination plant population was maintained at 3 plant per pot by thinning extra seedlings. After three weeks of sowing and growth, biochemical and physiological parameters were recorded, and data was analyzed statistically for significance.

Characterization of Selected Dry Region Zn Solubilizing Isolates Against Biochemical Attributes

The indole-3-acetic acid (IAA) production from dry region bacterial isolates was performed both in the absence and presence of L-tryptophan by colorimetric method using Salkowaki's method (Ehmann 1977). DF minimal salt broth media was inoculated with 48h old bacterial culture under both with and without L-tryptophan sets and incubated at 30 ± 1 °C for 48 h and 100 rpm. After 48h of incubation the broth culture was filtered using Whatman # 1 filter paper. Afterwards, 2 ml Salkowski's reagent and 1 ml culture aliquot were mixed and place in dark for 1h for color development and the absorbance was measured at 530 nm wavelength for known standards and sample and concentration was measured by comparing with standards curve. The oxidase and catalase activity of selected dry region rhizobacterial isolates was measured by following standard procedure of Cappuccino and Sherman (2002). Whereas, protease and cellulose degradation activity was measured by Chernin *et al*. (1998) and Sierra (1957), respectively. The assessment of ACC-deaminase activity was done by using the method of Penrose and Glick (2003) and Honma and Shimomura (1978). The qualitative Hydrogen cyanide (HCN) production was determined by picric acid dipped filters entrapment of HCN gas as described by Lorck (1948).

Root Colonization Ability of Dry Region ZSB

After seven days of germination in jar experiment wheat and maize plants were carefully uprooted in three replicate and gently washed with tap water. After surface disinfection of roots as described above the roots were washed with sterilized water for six time. About 200 mg of sterilized root was cut with seniors and ground in 5 mL sterilized distilled water in sterilized pestle and mortar. Afterwards the serial dilution method was adopted to obtain dilutions up to 10^{-6} and 1 mL from each dilution was spread on GPM agar plates and incubated for 48 hours at 30°C. After incubation that bacterial population was counted on digital colony counter (Digital S model, Selecta P) and CFU (colony forming unit) was calculated by following the formula given by Iqbal *et al*. (2020).

Statistical Analysis

After collecting the data of maize and wheat, data was analyzed statistically through one way analysis of variance (ANOVA) for significance following completely randomized design. Whereas significance of treatments was analyzed by least significant difference test (LSD) at 5% probability (Steel *et al*. 1997).

RESULTS

Isolation of Zn Solubilizing Isolates

Sixty isolates from wheat rhizosphere coded as IUB-26 to IUB-85 and sixty isolates from maize rhizosphere coded as IUB-86 to IUB-145 were isolated and screened for the Zn solubilizing quantitatively and qualitatively. Qualitative results of Zn solubilizing isolates revealed that 10 isolates from each maize and wheat have shown maximum solubilization of Zn (Table 1). The Zn solubilizing isolates of dry region from wheat and maize rhizosphere showed more efficient results qualitatively in term of Zn solubilization, solubilizing efficiency, solubilizing concentration and solubilizing index. From wheat rhizosphere isolate IUB-34 showed highest Zn solubilization diameter of halo zone (23 mm), solubilizing index (3.6), solubilizing efficiency (245.2%)

and from maize rhizosphere isolate IUB-96 showed maximum Zn solubilization diameter of halo zone (22 mm), solubilizing index (3.4), solubilizing efficiency (240.6%) as described in Table 2. Quantitative Zn solubilization confirmed that isolates IUB-34 and IUB-96 were performed excellent in Zn solubilization as 26.5 mg L^{-1} and 25.1 mgL^{-1}, respectively (Table 2). The isolates IUB-28, IUB-29, IUB-32, IUB-33, IUB-38, IUB-42, IUB-45, IUB-55, IUB-67, IUB-81 from wheat rhizosphere and IUB-87, IUB-90, IUB-94, IUB-95, IUB-109, IUB-115, IUB-116, IUB-125, IUB-126, IUB-133 from maize rhizosphere, were not selected due to poor Zn solubilization.

Table 1. Potential of dry region Zn solubilizing isolates for zinc solubilization, urease activity, phosphorus solubilization, exopolysaccharide and siderophore production.

Bacterial Isolates	Zinc Solubilization	Urease Activity	Phosphorus Solubilization	EPS Production	Siderophore Production
			Wheat		
IUB-27	++	++	++	++	++
IUB-28	+	+	+	+	+
IUB-29	+	+	+	+	+
IUB-30	++	++	++	++	++
IUB-32	+	+	+	+	+
IUB-33	+	+	+	+	+
IUB-34	++	++	++	++	++
IUB-38	+	+	+	+	+
IUB-41	++	++	++	++	++
IUB-42	+	+	+	+	+
IUB-45	+	+	+	+	+
IUB-47	++	++	++	++	++
IUB-50	++	++	++	++	++
IUB-55	+	+	-	+	+
IUB-57	++	++	++	++	++
IUB-60	++	++	++	++	++
IUB-67	+	+	-	+	+
IUB-70	++	++	++	++	++
IUB-80	++	++	++	++	++
IUB-81	+	+	-	+	+
			Maize		
IUB-87	+	+	-	+	+
IUB-88	++	++	++	++	++
IUB-90	+	+	+	+	+
IUB-93	++	++	++	++	++
IUB-94	+	+	+	+	+
IUB-95	+	+	+	+	+
IUB-96	++	++	++	++	++
IUB-100	++	++	++	++	++
IUB-103	++	++	++	++	++
IUB-107	++	++	++	++	++
IUB-109	+	+	-	+	+
IUB-111	++	++	++	++	++
IUB-115	+	+	-	+	+

IUB-116	+	+	+	+	+
IUB-117	++	++	++	++	++
IUB-119	++	++	++	++	++
IUB-125	+	+	-	+	+
IUB-126	+	+	+	+	+
IUB-132	++	++	++	++	++
IUB-133	+	+	+	+	+

Note: (++) indicated higher production (+) indicated lower production and negative sign (-) represent lack of production.

Table 2. The quantitative and qualitative Zn solubilization ability of dry region Zn solubilizing isolates (average of 3 replicates).

Bacterial Isolates (Zn)	HZD (mm)	SC (mgL^{-1})	SE (%)	SI
		Wheat		
IUB-27	13 de	20.3 e	201.9 g	3.10 cd
IUB-30	14 cd	21.8 d	208.4 f	3.10 cd
IUB-34	23 a	26.5 a	245.2 a	3.60 a
IUB-41	10 ef	17.1 f	191.5 i	2.90 d
IUB-47	17 bc	23.2 c	213.8 e	3.20 b-d
IUB-50	18 b	25.7 b	229.7 c	3.30 a-c
IUB-57	9.0 f	15.5 g	187.5 j	2.90 d
IUB-60	17 bc	23.5 c	219.9 d	3.20 b-d
IUB-70	13 de	17.5 f	196.8 h	3.0 cd
IUB-80	22 a	26.2 ab	236.2 b	3.50 ab
		Maize		
IUB-88	7.0 f	14.8 g	200.3 g	3.0 b-d
IUB-93	20.0 ab	26.3 a	204.9 f	3.03 bc
IUB-96	22.0 a	27.2 a	240.6 a	3.40 a
IUB-100	19.0 b	24.8 b	187.8 i	2.80 cd
IUB-103	15.0 cd	21.3 d	207.8 e	3.07 b
IUB-107	16.0 c	23.5 c	224.5 c	3.20 ab
IUB-111	15.0 cd	22.8 c	181.7 j	2.77 d
IUB-117	9.0 f	17.3 f	214.7 d	3.07 b
IUB-119	12.0 e	19.8 e	190.8 h	2.80 cd
IUB-132	13.0 de	20.5 de	231.4 b	3.37 a

Note: HZD= Holo-zone diameter, SC= Solubilizing concentration, SE= Solubilizing efficiency, SI= Solarization index.

In vitro Screening of ZSB Dry region Isolates Against Growth Promoting Traits

Zinc solubilizing isolates were screened for phosphorus solubilization, urease activity, siderophores and exopolysaccharide production. Results indicated in Table 3 that Zn solubilizing isolates of dry region isolated from wheat rhizosphere showed maximum phosphorus solubilization except IUB-55, IUB-67, IUB-81 and from maize rhizosphere IUB-87, IUB-109, IUB-115, IUB-125 (Table 1). Maximum halo zone diameter of wheat isolates was found in IUB-34 (22.3 mm) with P solubilizing efficiency (367.3%), P-concentration (33.4 mg L^{-1}) and solubilizing index (4.87). On the other hand, maize isolate IUB-96 showed maximum results in P solubilization in term of halo zone diameter, P-concentration, solubilizing index and solubilizing efficiency by 20.7 mm, 31.4 mgL^{-1}, 3.13, 201.7%, respectively. All the isolates of Zn solubilizing bacteria of dry region have the capability to produce urease activity, siderophores and EPS production (Table 1). Ten isolates from each wheat and maize rhizosphere tested positive traits like Zn and P solubilization, urease activity, siderophores, exopolysaccharide production for further evaluation.

Table 3. The quantitative and qualitative phosphorus solubilization ability of dry region Zn solubilizing isolates (average of 3 replicates).

Bacterial Isolates (P)	HZD (mm)	SC (mgL^{-1})	SE (%)	SI
		Wheat		
IUB-27	12.8 g	28.1 g	231.0 g	3.10 f
IUB-30	14.4 f	28.6 f	251.5 f	3.60 e
IUB-34	22.3 a	33.4 a	367.3 a	4.87 a
IUB-41	9.87 i	26.6 i	170.6 i	2.67 gh
IUB-47	16.0 e	30.2 e	195.8 e	3.83 d
IUB-50	18.4 c	31.9 c	340.5 c	4.23 c
IUB-57	8.27 j	25.5 j	141.9 j	2.53 h
IUB-60	17.3d	30.6 d	311.2 d	4.13 c
IUB-70	11.8 h	27.2 h	202.7 h	2.73 g
IUB-80	21.1 b	32.5 b	360.9 b	4.57 b
		Maize		
IUB-88	6.47 j	10.8 j	155.6 j	1.43 g
IUB-93	19.9 b	30.0 b	199.5 b	2.97 a
IUB-96	20.7 a	31.4 a	201.7 a	3.13 a
IUB-100	19.0 c	26.5 c	190.6 c	2.73 b
IUB-103	15.3 f	18.8 f	172.5 f	2.10 de
IUB-107	17.6 d	24.3 d	185.4 d	2.43 c
IUB-111	16.8 e	21.6 e	180.6 e	2.23 d
IUB-117	8.50 i	12.5 i	159.7 i	1.60 g
IUB-119	11.5 h	14.5 h	162.7 h	1.80 f
IUB-132	13.7 g	16.6 g	167.3 g	1.93 ef

HZD= Holo-zone diameter, SC= Solubilizing concentration, SE= Solubilizing efficiency, SI= Solarization.

Effect of Zn Solubilizing Isolates of Dry Region on the Growth of Wheat Plant

Under jar trial in axenic conditions, inoculation of Zn solubilizing isolates of dry region significantly improved the wheat growth (Table 4). Statistical analysis confirmed that maximum increase in shoot length was observed in strain IUB-34 that was 35.7% as compared to un-inoculated control followed by IUB-80, IUB-50 and IUB-60 strains that increase the length 30.4, 26.8, 23.7%, respectively. The maximum improvement of root length 32.2% increase was observed in strain IUB-34 followed by IUB-80, IUB-50, IUB-60 which was 29.4, 24.4, 21.5%, respectively, increased over un-inoculated control. Maximum shoot dry weight was also recorded in strain IUB-34 which showed 27.2% increase followed by IUB-80, IUB-50, IUB-60 that was 24.9, 22.3, 20.8% more than control, respectively. Moreover, IUB-34 showed significant improvement in root dry weight as compared to un-inoculated control that is 48%. Subsequent increase was observed in IUB-80, IUB-50 which was 43.5 and 39.5% respectively, over control.

The results demonstrated in Table 4 also depicted that the inoculation of dry region Zn solubilizing isolates improved wheat root colonization under axenic conditions. While the isolate IUB-34 has showed highest root colonization ability (61×10^5 cfu g^{-1} wheat root) followed by IUB-80, IUB-50 and IUB-60 that represented 56×10^5, 52×10^5 and 49×10^5 cfu g^{-1} wheat root respectively.

Table 4. Impact of dry region zinc solubilizing isolates inoculation on wheat growth and root colonization under control conditions (average of 3 replicates).

Treatments	Shoot Length	Root Length	Shoot Dry Weight	Root Dry Weight	Root Colonization
	(cm)		(g jar^{-1})		CFU × 10^5 mL^{-1}
Control	17.6 h	6.0 h	0.13 i	0.06 i	12 j
IUB-27	21.5 c-e	6.83 de	0.15 e	0.07 f	41 f
IUB-30	20.7 d-f	6.90 de	0.15 d	0.08 ef	43 ef
IUB-34	23.8 a	7.93 a	0.17 a	0.09 a	61 a
IUB-41	19.7 fg	6.37 fg	0.14 g	0.06 h	34 h
IUB-47	21.3 c-e	7.13 cd	0.15 d	0.08 de	45 e
IUB-50	22.3 bc	7.47 bc	0.16 c	0.08 bc	52 c
IUB-57	18.7 g	6.20 gh	0.13 h	0.06 hi	29 i
IUB-60	21.7 cd	7.30 c	0.16 c	0.08 cd	49 d
IUB-70	20.5 ef	6.57 ef	0.14 f	0.07 g	38 g
IUB-80	22.9 ab	7.77 ab	0.16 b	0.09 ab	56 b
LSD (p≤0.05)	1.0374	0.3663	2.489	3.520	2.8285
CV	1.69	1.81	0.57	1.63	2.32

Effect of Zn Solubilizing Isolates of Dry Region on Antioxidant Enzymes and Physiological Activity of Wheat Plant

Data regarding antioxidant enzymes activity represented that all the applied dry region Zn solubilizing isolates significantly improved antioxidant activities like SOD (superoxide dismutase), POD (peroxidase dismutase), POX (peroxidase), CAT (catalase), APX (ascorbate peroxidase) in wheat (Table 5). Data regarding SOD activity reveled that IUB-34 caused highest enzymatic activity that shown 19.2% as compared to control followed by IUB-80, IUB-50, IUB-60 stain with 17.3, 16.3, 15.5% higher SOD activities, respectively. Moreover, IUB-50 strain has caused maximum increase in POD activity that was 14.3% more than the control, followed by IUB-34 and IUB-80 which shown 13.0 and 11.9% more POD activities, respectively over control. While the significant increase in POX activity was observed in treatment IUB-80 that was 23.3% followed by IUB-34 strain which showed 20.9% increase in POX activity as compared to control. The dry region Zn solubilizing isolate IUB-80 showed highest improvement in CAT and APX activities that was 15.7 and 13.8% higher as compared to the un-inoculated control treatment.

The application of dry region Zn solubilizing isolates significantly improved the the chlorophyll a, b and carotenoid contents in wheat seedlings under axenic conditions (Table 5). The maximum increase in chlorophyll “a” was observe in IUB-34 isolate that was 24.5%, followed by IUB-80 and IUB-50 which showed 19.9 and 18.5% more chlorophyll a content, respectively. While the bacterial isolates IUB-60 and IUB-47 showed similar increase of 17.2% as compared to un-inoculated control. Results regarding chlorophyll “b” contents reveled that IUB-34 isolate also showed maximum improvement (16%) in chlorophyll b contents followed by IUB-80, IUB-50 and IUB-60 with 14.2, 12.4, 11.8% more chlorophyll b contents, respectively. Similar trend was observed under carotenoid contents as maximum increase (21.5%) was caused by IUB-34 isolate as compared to un-inoculated control, followed by IUB-80 and IUB-50 with 20.0 and 18.5% improvement, respectively, as compared to control.

Effect of Zn Solubilizing Isolates of Dry Region on the Growth of Maize Plant

The inoculation of dry region Zn solubilizing isolates in maize under axenic conditions, significantly increased maize growth (Table 6). Maximum increase in shoot length was observe in strain IUB-96 that was 39.9% as compared to un-inoculated control, followed by IUB-93, IUB-100 and IUB-107 strains with 36.2, 33.8 and 31.6% increased shoot lengths, respectively. The maximum improvement in root length (43.8%) was also observed under IUB-96 inoculation followed by IUB-93, IUB-100 and IUB-107 with 40.0, 35.7, 31.3% increase in root lengths, respectively, than un-inoculated control. Similarly, maximum increase in shoot dry weight (30%) was also recorded in strain IUB-96 application, followed by IUB-93 and IUB-100 that was 27.6 and 25.3%, respectively over un-inoculated control. IUB-96 application has caused maximum improvement in root dry weight (33.8%) as compared to un-inoculated control followed by IUB-93 and IUB-100 with 30.9 and 29.4% increase, respectively. In contrary IUB-88 has caused non-significant improvements in all growth parameters as compared to control.

Table 5. Impact of dry region zinc solubilizing isolates inoculation on antioxidant enzymes activity and chlorophyll contents of wheat seedlings under control conditions (average of 3 replicates).

Treatments	SOD	POD	POX	CAT	APX	Chlorophyll a	Chlorophyll b	Carotenoid
	(Units g^{-1} FW)					(µg g^{-1})		
Control	125 i	18.7 h	0.29 i	16.6 h	179 i	0.50 f	0.56 f	0.22 g
IUB-27	138 f	20.3 ef	0.31 e-g	18.3 de	192 e	0.58 cd	0.61 d	0.24 c-f
IUB-30	141 e	20.7 cd	0.32 d-f	18.2 e	195 d	0.59 bc	0.62 cd	0.24 b-e
IUB-34	149 a	21.1 ab	0.35 ab	18.6 c	201 b	0.63 a	0.65 a	0.26 a
IUB-41	133 g	20.1 f	0.30 g-i	17.2 g	183 h	0.56 d	0.59 e	0.23 e-g
IUB-47	142 de	20.8 cd	0.34 a-c	18.5 cd	189 f	0.59 bc	0.63 b-d	0.25 a-d
IUB-50	145 bc	21.4 a	0.33 b-d	18.9 b	199 bc	0.60 bc	0.63 bc	0.26 a-c
IUB-57	130 h	19.4 g	0.29 hi	17.3 g	185 gh	0.52 ef	0.58 ef	0.22 fg
IUB-60	144 cd	20.9 b-d	0.33 c-e	18.7 bc	199 c	0.59 bc	0.63 b-d	0.25 a-c
IUB-70	134 g	20.6 de	0.31 f-h	17.6 f	186 g	0.53 e	0.59 e	0.23 d-g
IUB-80	147 b	20.9 bc	0.35 a	19.2 a	204 a	0.60 b	0.64 ab	0.26 ab
LSD ($p \leq 0.05$)	2.0946	0.3048	0.0168	0.2640	2.0946	0.0209	0.0183	0.0197
CV	0.52	0.51	1.81	0.50	0.37	1.26	1.02	2.78

Table 6. Impact of dry region zinc solubilizing isolates inoculation on maize growth and root colonization under control conditions (average of 3 replicates).

Treatments	Shoot Length	Root Length	Shoot Dry Weight	Root Dry Weight	Root Colonization
	(cm)		(g jar^{-1})		CFU × 10^5 mL^{-1}
Control	32.5 k	11.5 j	0.13 i	0.11 h	16 j
IUB-88	35.2 j	12.5 i	0.14 h	0.11 g	21 i
IUB-93	44.3 b	16.1 b	0.16 ab	0.14 ab	45 b
IUB-96	45.5 a	16.5 a	0.16 a	0.14 a	49 a
IUB-100	43.5 c	15.6 c	0.16 bc	0.14 bc	43 bc
IUB-103	40.3 f	14.5 e	0.15 e	0.13 e	34 e
IUB-107	42.8 d	15.1 d	0.16 cd	0.14 c	41 c
IUB-111	40.9 e	14.7 e	0.16 d	0.13 d	37 d
IUB-117	37.2 i	13.1 h	0.14 g	0.11 g	24 h
IUB-119	37.9 h	13.5 g	0.14 fg	0.12 f	28 g
IUB-132	38.9 g	13.9 f	0.15 f	0.12 f	31 f
LSD ($p \leq 0.05$)	0.3902	0.3173	3.408	3.370	2.5401
CV	0.34	0.76	0.78	0.93	2.59

The results presented in Table 6 depicted that the application of dry region Zn solubilizing isolates significantly increased the maize root colonization ability as compared to un-inoculated control under axenic conditions. Maximum maize root colonization was observed under IUB-96 isolate application that was 49×10^5 cfu g^{-1} maize root and the subsequent improvement was observed in isolates IUB-93, IUB-100 and IUB-107 with 45×10^5, 43×10^5, 41×10^5 cfu g^{-1} maize root, respectively, over un-inoculated control. However minimum colonization (34×10^5 cfu g^{-1} maize root) was under IUB-88 isolate application but still higher than control (16×10^5 cfu g^{-1} maize root).

Effect of Zn Solubilizing Isolates of Dry Region on Antioxidant Enzymes and Physiological Activity of Maize Plant

Data presented in Table 7 revealed that application of dry region Zn solubilizing isolates significantly improved the antioxidant enzymes activity like SOD (superoxide dismutase), POD (peroxidase dismutase), CAT (catalase), POX (peroxidase), APX (ascorbate peroxidase) in maize seedlings under axenic conditions as compared to control. The application of IUB-96 caused maximum SOD activity that was 14.1% more as compared to control followed by IUB-93, IUB-100 and IUB-107 with 13.8, 13.0 and 12.5%, higher SOD activities, respectively. Similarly, highest POD activity was also observed under IUB-96 application that was 14.4% more tha control followed by IUB-93, IUB-100 and IUB-107 with 13.4, 11.2 and 10.1% higher SOD activities, respectively. The significant increase in CAT activity was also observed in treatment IUB-96 that was 15.2% higher than the uninoculated control followed by IUB-93 which showed 13.9% increase in CAT activity as compared to control. The Zn solubilizing isolate IUB-93 showed highest improvement (15.7) in POX activity as compared to un-inoculated control followed by IUB-100 and IUB-96 with 13.3 and 12.0% increased POX activities, respectively. Maximum APX activity was observed in IUB-100 isolate which was 14.7% higher followed by IUB-96 and IUB-93 strains with13.3 and 12.2% higher APX activities than the un-inoculated control.

Results presented in Table 7 reveled a significant increase in chlorophyll a, b and carotenoid contents of maize by the application of dry region Zn solubilizing isolates as compared to the un-inoculated control under axenic conditions. The maximum increase in chlorophyll “a” and “b” contents was observed under IUB-96 isolate application, which was 23.6 and 22.5% higher than the control, followed by IUB-93 (22.0 and 20.3% increase, respectively) and IUB-100 (19.2 and 18.1% increase, respectively) over un-inoculated control. Results regarding carotenoid contents in maize seedling showed maximum improvement under IUB-96 isolate which was 14.1% as compared to un-inoculated control followed by IUB-93 and IUB-100 with 13.4 and 12.1% increase in carotenoid contents, respectively.

Table 7. Impact of dry region zinc solubilizing isolates inoculation on antioxidant enzymes activity and chlorophyll contents of maize seedlings under control conditions (average of 3 replicates).

Treatments	SOD	POD	POX	CAT	APX	Chlorophyll a	Chlorophyll b	Carotenoid
	(Units g^{-1} FW)					(µg g^{-1})		
Control	125 h	0.92 h	0.28 f	7.70 g	120 h	0.61 h	0.61 g	0.99 i
IUB-88	130 g	0.94 g	0.28 ef	7.93 f	123 g	0.63 h	0.63 f	1.02 h
IUB-93	143 ab	1.05 a	0.32 a	8.77 ab	135 bc	0.74 ab	0.72 ab	1.13 ab
IUB-96	143 a	1.06 a	0.31 a-c	8.87 a	136 ab	0.75 a	0.74 a	1.13 a
IUB-100	142 a-c	1.03 b	0.31 ab	8.70 ab	138 a	0.73 a-c	0.72 bc	1.11 bc
IUB-103	138 d	0.99 d	0.30 a-d	8.33 d	132 de	0.69 d-f	0.70 cd	1.09 de
IUB-107	141 bc	1.02 bc	0.31 a-c	8.60 bc	134 b-d	0.72 b-d	0.71 bc	1.11 b-d
IUB-111	140 c	1.01 cd	0.30 b-e	8.40 cd	133 c-e	0.71 c-e	0.70 c	1.10 c-e
IUB-117	132 fg	0.95 fg	0.29 d-f	8.03 ef	125 fg	0.66 g	0.65 ef	1.06 g
IUB-119	133 ef	0.96 ef	0.29 c-f	8.20 de	128 f	0.68 fg	0.65 e	1.07 fg
IUB-132	134 e	0.97 e	0.30 b-e	8.23 de	131 e	0.68 e-g	0.68 d	1.08 ef
LSD ($p\leq0.05$)	1.9008	0.0168	0.0175	0.2328	2.5401	0.0244	0.0216	0.0176
CV	0.48	0.58	2.01	0.96	0.67	1.21	1.08	0.56

Data shown three replicates mean. Within column same letter(s) followed by means are statistically not differ significantly as per LSD test at $p\leq0.05$.

Characterization of Dry Region Zn Solubilizing Isolates

After jar trial under axenic conditions four (two from wheat and two from maize trial) best Zn solubilizing rhizobacterial isolates of dry region performed best were further evaluated for in vitro plant growth promoting characters. The data represented in Table 8 showed the *in vitro* characterization of selected bacterial isolates. All the tested isolates were positive for hydrogen cyanide production (HCN), protease activity, cellulose degradation activity and catalase activity. Moreover, the ACC-deaminase activity was also found in all tested bacterial isolates, while the IUB-34 and IUB-96 strains showed positive oxidase activities whereas IUB-80 and IUB-93 were found negative for oxidase activities. Indole-3-acetic acid (IAA) production was also positive in all isolates in the presence or absence of its precursor L-tryptophan. IUB-34 isolate showed maximum IAA production (24 μg mL^{-1}) in the presence of L-tryptophan. However, the strains IUB-80, IUB-93 and IUB-96 shown similar IAA production with L-tryptophan which was 19 μg mL^{-1}. On the other hand, without L-tryptophan maximum results was recorded in IUB-96 bacterial isolate which produced 15 μg mL^{-1} IAA followed by IUB-34 and IUB-93 with both producing 14 μg mL^{-1} IAA and IUB-80 strain showed minimum (12 μg mL^{-1}) IAA production in the absence of L-tryptophan.

Table 8. Characterization of selected isolates of dry region zinc solubilizing bacteria for improving plant growth and biochemical attributes.

Characters		IUB-34	IUB-80	IUB-93	IUB-96
Hydrogen cyanide production (HCN)		+	+	+	+
Protease activity		+	+	+	+
Cellulose degradation activity		+	+	+	+
Catalase activity		+	+	+	+
ACC-deaminase activity		+	+	+	+
Oxidase activity		+	-	-	+
IAA production (μg mL^{-1})	With L-tryptophan	24	19	19	19
	Without L-tryptophan	14	12	14	15

Positive sign (+) represents the availability of character and negative sign (-) represent lack of character.

DISCUSSION

Zinc plays a crucial role as a micronutrient, contributing significantly to metabolic and physiological functions in both humans and plants (Natasha *et al*. 2022). Worldwide, especially in developing nations, Zn deficiency is prevalent among plants and humans, because of crop cultivation in Zn deficient soils (Zia *et al*. 2020). Different techniques were used to reduce the Zn deficiency in maize and wheat crop and from this application of Zn solubilizing isolates to improve the Zn bio-availability is the most effective and environmentally friendly way. The study was executed to isolate the dry region bacteria from the rhizosphere of wheat and maize crops growing in desert region of Bahawalpur and their role in growth promotion of wheat and maize crop by enhancing the availability of zinc. The isolates were tested for their ability to solubilize the Zn by using zinc oxide as an insoluble source. In this study, results reveled that out of 60 isolates only 20 isolates showed the zinc solubilization ability. The findings of our research work are in line with the Prathap *et al*. (2022) and Bhakat *et al*. (2021), who find the differential Zn-solubilization ability in bacterial isolates. Rhizobacterial isolates from wheat rhizosphere IUB-34, IUB80 and IUB-50 showed better zinc solubilization results. Inoculation of Zn solubilizing isolates produces organic acids, chelating agents which helps in enhancing the zinc availability in soils for longer periods (Masood *et al*. 2022).

Moreover, the dry region Zn solubilizing isolates possessed several plant growth promoting attributes. Results confirmed that isolates possess Zn-solubilization also solubilize the insoluble tri-calcium phosphate except the bacterial isolates IUB-55, IUB-67 and IUB-81. Our results are in line with the results of Ahmad *et al*. (2018), Bumunang and Babalola (2014) and Naseer *et al*. (2020). The exopolysaccharides production by bacteria is also considered a growth promoting character which is helpful in root colonization, drought tolerance, nutrients uptake, biofilm formation and protection against pathogens (Vanderlinde *et al*. 2010; Mahmood *et al*. 2024). Gontia-Mishra *et al*. (2017) and Mumtaz *et al*. (2017) also confirmed the similar aspects of Zn solubilizing isolates through exopolysaccharides production. Urease activity by the Zn solubilizing isolates also correlated with the release and uptake of N in soil as described by Mumtaz *et al*. (2017) that *Bacillus aryabhattai* isolates S10 and ZM31 with positive urease activity as growth promoters. The results of siderophore production are in line with the findings of Dinesh *et al*. (2018) and Hussain *et al*. (2015) which results in fortification of Zn in crops (Aimen *et al*. 2024).

The axenic conditions jar trial results showed that inoculation with dry region Zn solubilizing rhizobacterial isolates enhance growth parameters of wheat and maize, our findings are in line with Hussain *et al*. (2015), which depicted the application of Zn solubilizing bacteria have ability to improve shoot, root length, shoot, root fresh biomass, shoot, root dry biomass and also total dry biomass. The improvement in growth parameters might be due to the improvement in nutrients availability and the synthesis of growth regulators for plants by PGPRs (Ahmad *et al*. 2020). The increases in root length and plant biomass of wheat, due to Zn solubilizing bacteria was also described by the Javed *et al*. (2018). Mumtaz *et al*. (2017), also discussed that the Zn solubilizing bacteria can increase the root, shoot growth and their total biomass in maize plant.

The significant improvement in antioxidant activities; SOD (superoxide dismutase), POD (peroxidase dismutase), POX (peroxidase), APX (ascorbate peroxidase), and CAT (catalase) in the seedlings of both crops might be due to the improvement in nutrient availability, hormonal balance, like Zn to crop plants by the activity of Zn solubilizing bacteria (Anwar *et al*. 2021). The results of our findings are like Charaborty *et al*. (2013), revealed that the use of *Bacillus* sp. (*B. safensis*) enhances the activity of antioxidant enzymes in the leaves of crop plants. Moreover, the higher antioxidant activities might be due to the physiological regulations of the wheat and maize by the application of the dry region ZSB (Ain *et al*. 2020; Efthimiadou *et al*. 2020).

Additionally, the application of Zn solubilizing isolates leads to increased photosynthetic pigmentation (chlorophyll a, b, and carotenoids) in both wheat and maize crops which were in line with the findings of Hussain *et al*. (2015). The improvement in chlorophyll and carotenoid contents might be due to the improvement in growth attributes especially enhanced nitrogen uptake in plant and other nutrients (Dar *et al*., 2020). The results of our study are accordance with findings of Kang *et al*. (2014) which also reported similar results as the use of Zn solubilizing isolates significantly increase the chlorophyll contents in maize seedlings. Results regarding improvement in root colonization by applying dry region Zn solubilizing isolates are in line with the finding of Rezaee-Niko *et al*. (2018), who reported that inoculation of Zn solubilizing isolates with seed improve the root colonization which ultimately improve the plant growth. Root colonization also increase the nutrient uptake and improve plant growth (Iqbal *et al*. 2020).

Zinc solubilizing bacterial isolates were further characterized for IAA production ability with and without L-tryptophan and these results are in line with the findings of Ramesh *et al*. (2014), Ahmad *et al*. (2013), Mumtaz *et al*. (2017) and Dar *et al*. (2023) which depicted that rhizobacterial strains boost the auxin production by supplying L-tryptophan in the growth medium as compared to without L-tryptophan. Production of IAA by the rhizobacteria in the wheat and maize rhizosphere is responsible for better root proliferation which can enhance the nutrients status and water contents in plants under both stressful and non-stressful conditions (Atajan and Zohan, 2020; Figueredo *et al*. 2023; Voronina *et al*. 2023).

All the strains have positive ability of cellulose degradation, catalase and protease activity in plate assay. Iqbal *et al*. (2020) reported comparable results, observing enhanced protease and catalase activity in several endophytic and rhizobacterial strains. They also detailed the cellulose degradation capability of *Bacillus* sp. These enzymes have ability to improve crop growth under stressful conditions especially biotic stresses (Vaishnay *et al*. 2020). The results of hydrogen cyanide (HCN) production by these bacteria are in line with the findings of Mumtaz *et al*. (2017) which also described the positive HCN production in *Bacillus aryabhattai* isolate S10 and ZM31 and Shoukry *et al*. (2018) who described that the HCN, oxidase and siderophore production in wheat and maize rhizosphere by Zn solubilizing isolates is a defense mechanism against crop pathogens. Moreover, the PGPRs with ACC-deaminase activity are helpful for plants to ameliorate abiotic stress induced elevated ethylene levels and improve plant growth by hydrolyzing ACC (the precursor of ethylene) into ammonia and α-ketobutarate (Arshad *et al*. 2007).

CONCLUSION /FUTURE DIRECTIONS

In conclusion we can say that dry region Zn solubilizing isolates IUB-34, IUB-80, IUB-93 and IUB-96 showed promising results in vitro screening (Zn solubilization), growth improvement of maize and wheat under axenic conditions, and possession of plant growth promoting attributes. Future research should focus on the following aspects:

1- The studied isolates should be explored in terms of mechanism of action, compatibility, consortium application in natural conditions.
2- The molecular identification and development of suitable biofertilizer for sustainable cereals production and Zn biofortification should be developed to cope with the food security and malnutrition.

AUTHORS CONTRIBUTION

In design and commencement of research work all the authors contribute their knowledge. Plan of experiment, preparation of material, collection and analysis of data and tables preparation were carried out by Muhammad Umair Asghar, Azhar Hussain, Hammad Anwar, Qudsia Nazir, Abubakar Dar, Hafiz Tanvir Ahmad, Nadeem Tariq and Muhammad Usman Jamshaid. Manuscript initial draft prepared by Muhammad Umair Asghar and Hammad Anwar and further edited by Azhar Hussain and Hafiz Tanvir Ahmad. All authors checked the paper draft, comment on them and finalized the draft.

CONFLICT OF INTEREST

The authors confirm that the research was conducted without any financial or commercial contribution that might be seen as a possible conflict of interest.

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