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ORIGINAL RESEARCH ARTICLE

Indigenous zinc-solubilizing bacterial consortium enhances seedling establishment, root growth and grain yield of field-grown wheat

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ABSTRACT

Zinc (Zn) deficiency limits wheat productivity on the calcareous, low-organic-matter soils of southern Punjab, Pakistan, where applied Zn is rapidly fixed. Zinc-solubilizing bacteria (ZSB) may mobilize native soil Zn, but field comparisons of ZSB consortia with their component isolates are scarce. *Two indigenous* ZSB isolates (BS-1, BS-2) from Rahim Yar Khan were applied as seed coatings, singly and as a consortium, to cultivars Akbar-19 and Nawab-21 in a 2024–2025 field trial (randomized complete block design, four replicates). They were compared with recommended chemical fertilization, a non-Zn-solubilizing isolate and an uninoculated control. Data on 19 germination, growth, root and yield traits were analysed by two-way ANOVA and Tukey's HSD test. Treatment and cultivar effects were significant ($P < 0.001$) for all traits; the cultivar × treatment interaction was significant only for seedling fresh weight and grain yield. *The consortium ranked* first for every trait, raising germination from 70 to 92% (Akbar-19) and from 78 to 96% (Nawab-21), shortening mean germination time by 1.2–1.3 d, and increasing root dry weight by 155–214%, 1000-grain weight by 42–50% and grain yield by 125–153% over the control. Its grain yield (3.8 and 4.5 g plant^{-1}) exceeded that of the best single isolate by 45–65% and of chemical fertilization by 29–36%, whereas the non-solubilizing isolate gave only 15–20% gains. An indigenous two-strain ZSB consortium outperformed its single isolates and chemical fertilization under semi-arid field conditions. Grain Zn analysis, isolate identification and multi-season trials are needed before it can be recommended for bio fortification.

KEYWORDS

Triticum aestivum; zinc-solubilizing bacteria; microbial consortium; seed inoculation; plant growth-promoting rhizobacteria; bio fortification; semi-arid agriculture

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1. Introduction

Wheat (*Triticum aestivum* L.) is the most important food crop in Pakistan and a major source of dietary energy, protein and minerals for most of the population [1]. Wheat grain is inherently low in zinc (Zn) and rich in phytate, which further limits Zn absorption; populations that depend heavily on wheat-based diets are therefore vulnerable to Zn malnutrition [2]. Globally, an estimated 17% of people are at risk of in adequate Zn intake, with South Asia among the most affected regions [3]. In Pakistan, the National Nutrition Survey 2018 found Zn deficiency in 22.1% of women of reproductive age and 18.6% of children under five [4], and food-supply analyses place the national risk of dietary Zn deficiency at about one-quarter of the population [5, 6]. Zinc deficiency impairs child growth and immune function and contributes to stunting, so increasing the Zn supplied by staple cereals is a public-health priority as well as an agronomic one.

In plants, Zn is a structural or catalytic component of thousands of proteins, including enzymes of carbohydrate metabolism, protein synthesis, membrane integrity and auxin bio synthesis [7]. Zinc-deficient wheat shows reduced tillering, smaller leaves, poor root growth and lower grain set, and grain from Zn-deficient fields produces seedlings of lower vigour [2]. Zinc deficiency is the most widespread micronutrient disorder of crops: about half of the world's cereal-growing soils have low plant-available Zn, mostly because of high pH, free calcium carbonate, low organic matter and high bicarbonate and phosphate concentrations rather than low total Zn [2, 8]. These conditions are typical of the irrigated, alkaline, calcareous soils of southern Punjab. Rahim Yar Khan district has a hot desert climate with a mean annual temperature of about 26 °C and roughly 100 mm of annual rainfall [9], so wheat depends entirely on irrigation and is often grown after cotton on soils low in organic matter.

Agronomic bio fortification with Zn fertilizers can raise both yield and grain Zn, particularly when Zn is applied to foliage [10, 11]. Field programmes in Pakistan and India have shown that combining Zn-efficient cultivars with Zn fertilization increases grain Zn concentration [12, 13], and economic modelling indicates that wider Zn fertilizer use in Pakistan would be highly cost-effective [5]. However, adoption remains limited, and most soil-applied Zn is rapidly converted to sparingly soluble carbonates, phosphates and hydroxides, so its recovery by the crop is low and responses vary strongly between sites [6, 14]. Approaches that increase the availability of native and residual soil Zn would therefore complement fertilization, especially for resource-poor farmers.

Zinc-solubilizing bacteria (ZSB) are rhizosphere microorganisms that convert insoluble Zn compounds into plant-available forms. They do so mainly by excreting organic acids such as gluconic and 5-ketogluconic acid, which lower rhizosphere pH and chelate Zn, and also by proton extrusion and by producing siderophores and other ligands [15-17]. Many ZSB also have other plant growth-promoting traits, including indole-3-acetic acid (IAA) production, phosphate solubilization and nitrogen fixation, which stimulate root development and nutrient acquisition independently of Zn supply [18-21]. Inoculation of wheat with ZSB has increased growth, yield and grain or tissue Zn in pot and field studies [22-26], partly through up-regulation of ZIP-family Zn transporters in roots [27, 28]. Field trials in Pakistan have shown that ZSB enhance the response of wheat to applied $ZnSO_4$ in calcareous soil [29] and that bacteria integrated with compost and mineral Zn improve yield, grain Zn and soil health, including in the arid Bahawalpur division that adjoins Rahim Yar Khan [30, 31].

Single-strain inoculants often perform inconsistently in the field because introduced strains must compete with the native microflora and survive heat, drought and salinity [32]. Combining compatible strains with complementary traits in a consortium is one strategy to improve reliability [33 34]. In wheat, consortia of ZSB or mineral-solubilizing bacteria have given larger growth or Zn responses than individual isolates in glasshouse and pot studies [33-35]. The advantage of consortia is, however, not universal and appears to depend on environmental conditions; in tomato, for example, a consortium outperformed single strains in a desert field but not in a favourable greenhouse [36]. Isolates obtained from the target environment may also be better adapted to its soil and climate than strains introduced from elsewhere.

Despite this progress, three gaps remain. First, most comparisons of ZSB consortia with their component strains in wheat have been made in sand culture, pots or glasshouses rather than under farmers' field conditions. Second, few studies have tested ZSB isolated from the hot, arid, calcareous wheat–cotton zone of southern Punjab on the cultivars currently grown there. Third, inoculation studies rarely include a bacterial control that lacks Zn-solubilizing activity, so the specificity of the response to the selected trait is seldom tested. To address these gaps, we isolated ZSB from wheat fields in Rahim Yar Khan and evaluated two isolates, singly and as a consortium, on two recently released cultivars, Akbar-19 and Nawab-21, under field conditions. The treatments also included a recommended chemical-fertilizer comparator and a non-Zn-solubilizing bacterial isolate. We hypothesized that (i) seed inoculation with indigenous ZSB would improve germination, seedling vigour, root development and yield formation relative to the uninoculated control and to the non-solubilizing isolate; (ii) the two-strain consortium would outperform either isolate alone; and (iii) the direction of the treatment response would be consistent across the two cultivars.

2. Materials and methods

2.1 Study site and plant material

The field experiment was conducted during the 2024–2025 *rabi* (winter) season at Karamabad, District Rahim Yar Khan (approximately 28.4° *N*, 70.3° E), southern Punjab, Pakistan. The district has a hot desert climate with a mean annual temperature of about 26 °C and roughly 100 mm of annual rainfall [9]; the wheat crop was therefore fully irrigated. Soils of the area are alkaline and calcareous, conditions that restrict Zn availability to crops [8 31]. Two bread-wheat cultivars approved for cultivation in Punjab, Akbar-19 and Nawab-21, were used. Seed was sown in November at 125 kg ha^{-1} (50 kg $acre^{-1}$), following provincial recommendations for timely sown irrigated wheat [37,38], in rows 22.5 cm apart. Land preparation, irrigation, weeding and plant protection followed local recommendations and were applied uniformly to all plots.

2.2 Isolation and screening of zinc-solubilizing bacteria

Rhizosphere soil (0–15 cm) was collected from wheat fields in Rahim Yar Khan and transported aseptically to the laboratory. Serial dilutions in sterile distilled water were spread on modified Pikovskaya agar (g L^{-1}: glucose 10.0; ammonium sulfate 0.5; potassium chloride 0.2; $MgSO_4 \cdot {}_7H_2O$ 0.1; $MnSO_4 \cdot H_2O$ 0.002; $FeSO_4 \cdot {}_7H_2O$ 0.002; yeast extract 0.5; agar 15.0; pH 7.0 ± 0.2) in which the insoluble mineral was replaced by 5.0 g L^{-1} insoluble ZnO as the sole Zn source [15,33], and incubated at 28 ± 2 °C for 48–72 h. Colonies producing clear halos were purified by repeated streaking and maintained on nutrient-agar slants at 4 °C. The Zn solubilization index was calculated as the ratio of halo diameter to colony diameter. The two isolates with the largest halos (BS-1 and BS-2) were selected, together with one isolate that grew on the medium without forming a halo, which served as a non-Zn-solubilizing bacterial comparator (T_5). The isolates were characterized phenotypically by their Zn-solubilizing activity only; molecular identification was beyond the scope of this field evaluation and is reported as a limitation.

2.3 Inoculum preparation and seed treatment

Each isolate was grown in nutrient broth at 28 °C and 120 rpm for 48 h and adjusted to approximately 10^8 CFU mL^{-1}. For the consortium, equal volumes of the two adjusted suspensions were mixed, so the total cell density applied to seed matched that of the single-isolate treatments. Seeds were surface-disinfected with 1% sodium hypochlorite for 2–3 min, rinsed repeatedly in sterile distilled water and coated with the respective suspension (10 mL per 100 g seed) using 10% sucrose solution as adhesive, then shade-dried for 30 min before sowing. Control seed received sterile broth and adhesive only.

2.4 Experimental design and treatments

Six treatments (Table 1) were tested on both cultivars in a two-factor (cultivar × treatment) experiment laid out as a randomized complete block design (RCBD) with four replications, giving 48 plots; treatments were randomized independently within each block. In the chemical-fertilizer treatment (T_4), nitrogen, phosphorus and potassium were applied at the provincial recommendation for medium-fertility soils (42–34–25 kg N–P_2O_5–K_2O $acre^{-1}$, ≈ 104–84–62 kg ha^{-1}), with all P and K and one-third of N at sowing and the remaining N top-dressed in splits with the early irrigations, together with $ZnSO_4 \cdot H_2O$ (33% Zn) at 6 kg $acre^{-1}$ (≈ 15 kg ha^{-1}) at sowing [37,38]. Inoculated treatments (T_1–T_3, T_5) and the control received no fertilizer, so that bacterial effects could be assessed against the unamended soil.

Table 1. Treatments applied to wheat cultivars Akbar-19 and Nawab-21.

Code	Treatment	Description
T_1	BS-1	Seed coated with zinc-solubilizing isolate BS-1 (≈10^8 CFU mL^{-1})
T_2	BS-2	Seed coated with zinc-solubilizing isolate BS-2 (≈10^8 CFU mL^{-1})
T_3	Consortium	Seed coated with an equal-volume mixture of BS-1 and BS-2
T_4	Chemical fertilizer	Recommended NPK + $ZnSO_4$, no inoculation
T_5	Non-ZSB isolate	Seed coated with a bacterial isolate lacking zinc-solubilizing activity
T_6	Control	Uninoculated seed treated with sterile broth and adhesive only

2.5 Germination and seedling vigour

Treated seeds of each cultivar were germinated on two layers of filter paper (Whatman No. 1) moistened with 5 mL sterile distilled water in 9-cm Petri dishes, 25 seeds per dish, at 25 ± 2 °C for 8 days, with four replicate dishes per treatment. Germinated seeds (radicle ≥ 2 mm) were counted daily. Germination percentage (GP) = 100 × germinated seeds/total seeds; mean germination time (MGT) = $\Sigma(n_i t_i)/\Sigma n_i$, where n_i is the number of seeds germinating on day t_i [39]; germination index (GI) = $\Sigma(G_t/T_t)$, where G_t is the number of seeds germinated on day T_t, following the Association of Official Seed Analysts (AOSA) method. Radicle and plumule lengths were measured on ten seedlings per replicate at the end of the test; fresh weight was recorded after blotting, and dry weight after oven-drying at 70 °C for 72 h.

2.6 Growth, root and yield measurements

Plant height was measured from the soil surface to the tip of the uppermost leaf, and productive tillers were counted on ten randomly selected plants per plot. *Leaf area* was estimated as leaf length × maximum width × 0.75, a correction factor for the non-rectangular shape of wheat leaves, and summed over all green leaves of the main shoot at the vegetative stage. Vegetative biomass was determined at the same stage after oven-drying above-ground tissue at 70 °C to constant weight. Roots were sampled at the vegetative stage, excavated carefully, washed free of soil and measured for length (collar to tip), volume (water displacement) and dry weight (70 °C, 72 h). At maturity, spike length and spikelets per spike were recorded; plants were harvested and threshed, and grain yield and biological yield (grain + straw) were expressed per plant. 1000-grain weight was determined from counted samples of sun-dried, fully developed grain (approximately 10–12% moisture).

2.7 Statistical analysis

Data were analysed by two-way analysis of variance with cultivar, treatment and their interaction as fixed effects using CoStat 6.303 (CoHort Software, Monterey, CA, USA) [40]. Variation among replicate blocks was not partitioned from the residual, which was therefore based on 36 degrees of freedom; pooling block variation into the error term makes the F-tests for treatment and cultivar conservative. Where the treatment effect was significant, means were compared within each cultivar by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$ using the pooled error mean square; the standard error of each cultivar × treatment mean was calculated as $\sqrt{(MS_{error}/4)}$. Relative effects were calculated as 100 × (treated – control)/control.

3. Results

3.1 Analysis of variance

Treatment and cultivar main effects were highly significant ($P < 0.001$) for all 19 traits (Table 2). The cultivar × treatment interaction was significant only for seedling fresh weight ($P = 0.001$) and grain yield ($P = 0.003$); for all other traits the ranking and relative magnitude of treatment effects were similar in the two cultivars. Nawab-21 exceeded Akbar-19 for every trait and germinated faster.

Table 2. Summary of two-way ANOVA (*F* values) for germination, growth and yield traits of two wheat cultivars inoculated with zinc-solubilizing bacteria.

Trait	Cultivar (C), df = 1	Treatment (*T*), df = 5	C × *T*, df = 5	Error MS, df = 36
Germination (%)	164.2 ***	150.4 ***	1.27 ns	2.93
Mean germination time	180.5 ***	251.7 ***	0.50 ns	0.00667
Germination index	340.4 ***	336.5 ***	0.51 ns	0.243
Radicle length	395.2 ***	346.9 ***	0.81 ns	0.0281
Plumule length	560.0 ***	514.0 ***	1.01 ns	0.0292
Seedling fresh weight	470.9 ***	468.8 ***	5.28 ***	0.000609
Seedling dry weight	288.0 ***	286.4 ***	1.20 ns	0.0000667
Plant height	250.6 ***	296.1 ***	0.19 ns	2.60
Productive tillers	67.6 ***	69.8 ***	0.40 ns	0.208
Leaf area	475.9 ***	499.6 ***	0.61 ns	6.09
Vegetative biomass	584.6 ***	635.0 ***	2.11 ns	0.112
Root length	288.0 ***	304.4 ***	1.80 ns	0.667
Root volume	220.5 ***	200.1 ***	0.90 ns	0.667
Root dry weight	338.0 ***	387.6 ***	2.00 ns	0.00667
Spike length	2964.5 ***	2965.7 ***	1.70 ns	0.00667
Spikelets per spike	284.1 ***	240.5 ***	0.94 ns	0.319
1000-grain weight	658.2 ***	733.7 ***	0.36 ns	0.336
Grain yield	760.5 ***	886.1 ***	4.50 **	0.00667
Biological yield	481.8 ***	526.8 ***	2.14 ns	0.246

*** $P < 0.001$; ** $P < 0.01$; ns, not significant ($P \geq 0.05$). MS, mean square.

3.2 Germination and seedling vigour

All bacterial treatments improved germination relative to the control, but the magnitude depended strongly on the isolate (Table 3). *The consortium gave* the highest germination in both cultivars (92% and 96%, versus 70% and 78% in controls) and the shortest mean germination time (3.5 and 3.2 d, versus 4.8 and 4.4 d), giving germination indices 51–57% above the control. In Nawab-21, consortium germination did not differ significantly from chemical fertilization, but GI, MGT and seedling traits did. Radicle and plumule lengths under the consortium were 50–64% and 47–62% greater than the control, respectively, and seedling dry weight more than doubled. The single isolates gave intermediate values, with BS-2 generally ranking above BS-1, while the non-solubilizing isolate (T_5) differed only modestly from the control (germination +5 to +7%). The significant interaction for seedling fresh weight reflected a larger consortium effect in Nawab-21 in absolute terms (+0.56 g versus +0.50 g), without any change in treatment ranking.

Table 3. Germination and seedling vigour of wheat cultivars under zinc-solubilizing bacterial treatments.

Treatment	GP (%)	MGT (d)	GI	Radicle (cm)	Plumule (cm)	Fresh wt (g)	Dry wt (g)
Akbar-19							
BS-1 (T_1)	82 ^{c}	4.2 ^{d}	18.6 ^{c}	6.2 ^{c}	8.1 ^{d}	0.85 ^{c}	0.12 ^{c}
BS-2 (T_2)	84 ^{bc}	4.0 ^{c}	19.6 ^{c}	6.5 ^{c}	8.5 ^{c}	0.89 ^{c}	0.13 ^{c}
Consortium (T_3)	92 ^{a}	3.5 ^{a}	24.2 ^{a}	8.2 ^{a}	10.5 ^{a}	1.15 ^{a}	0.20 ^{a}
Chemical fertilizer (T_4)	87 ^{b}	3.8 ^{b}	20.8 ^{b}	7.0 ^{b}	9.1 ^{b}	0.97 ^{b}	0.16 ^{b}
Non-ZSB isolate (T_5)	75 ^{d}	4.5 ^{e}	16.7 ^{d}	5.5 ^{d}	7.2 ^{e}	0.75 ^{d}	0.09 ^{d}
Control (T_6)	70 ^{e}	4.8 ^{f}	15.4 ^{e}	5.0 ^{e}	6.5 ^{f}	0.65 ^{e}	0.07 ^{e}
Nawab-21							
BS-1 (T_1)	88 ^{c}	3.9 ^{d}	21.3 ^{c}	7.1 ^{d}	9.2 ^{d}	0.95 ^{d}	0.15 ^{d}
BS-2 (T_2)	91 ^{bc}	3.7 ^{c}	22.6 ^{b}	7.6 ^{c}	9.8 ^{c}	1.05 ^{c}	0.17 ^{c}
Consortium (T_3)	96 ^{a}	3.2 ^{a}	26.9 ^{a}	9.0 ^{a}	11.5 ^{a}	1.35 ^{a}	0.25 ^{a}
Chemical fertilizer (T_4)	93 ^{ab}	3.5 ^{b}	23.5 ^{b}	8.0 ^{b}	10.2 ^{b}	1.17 ^{b}	0.20 ^{b}
Non-ZSB isolate (T_5)	82 ^{d}	4.2 ^{e}	19.0 ^{d}	6.5 ^{e}	8.4 ^{e}	0.88 ^{e}	0.13 ^{e}
Control (T_6)	78 ^{e}	4.4 ^{f}	17.8 ^{e}	6.0 ^{f}	7.8 ^{f}	0.79 ^{f}	0.11 ^{f}
SE (n = 4)	0.9	0.04	0.25	0.08	0.09	0.012	0.004
Tukey HSD (0.05)	3.6	0.17	1.05	0.36	0.36	0.052	0.017

Values are means (n = 4). Within each cultivar and column, means sharing a letter do not differ at $P \leq 0.05$ (Tukey's HSD); for MGT, "a" denotes the fastest germination. GP, germination percentage; MGT, mean germination time; GI, germination index.

3.3 Shoot growth, biomass and root development

Plant height, productive tillers, leaf area and vegetative biomass followed the same treatment order: consortium > chemical fertilizer > BS-2 ≥ BS-1 > T_5 > control (Table 4). Consortium-inoculated plants were 39–47% taller than control plants and carried 5.75 and 6.75 productive tillers per plant, compared with 2.00 and 3.00 in the controls. *Leaf area* and vegetative biomass increased by 46–52% and 74–82%, respectively.

Root traits were the most responsive vegetative characters (Fig. 2). Under the consortium, root length reached 33 and 37 cm (+61 to +74%), root volume 22 and 25 cm^3 (+79 to +120%) and root dry weight 2.2 and 2.8 g (+155 to +214%). Root dry weight under the consortium also exceeded that under chemical fertilization by 40–38%, whereas shoot height exceeded fertilization by only 8–9%, indicating a preferential stimulation of root growth by the bacterial consortium.

Table 4. Shoot growth, vegetative biomass and root traits of wheat cultivars under zinc-solubilizing bacterial treatments.

Treatment	Height (cm)	Tillers plant⁻¹	Leaf area (cm^2)	Veg. biomass (g)	Root length (cm)	Root volume (cm^3)	Root dry wt (g)
Akbar-19							
BS-1 (T_1)	68 ^{d}	3.75 ^{c}	125 ^{c}	12.5 ^{c}	24 ^{d}	15 ^{c}	1.2 ^{d}
BS-2 (T_2)	72 ^{c}	3.75 ^{c}	130 ^{c}	13.2 ^{c}	26 ^{c}	16 ^{c}	1.4 ^{c}
Consortium (T_3)	85 ^{a}	5.75 ^{a}	160 ^{a}	17.8 ^{a}	33 ^{a}	22 ^{a}	2.2 ^{a}
Chemical fertilizer (T_4)	78 ^{b}	4.75 ^{b}	138 ^{b}	14.6 ^{b}	28 ^{b}	18 ^{b}	1.6 ^{b}
Non-ZSB isolate (T_5)	62 ^{e}	2.75 ^{d}	112 ^{d}	10.8 ^{d}	21 ^{e}	12 ^{d}	0.9 ^{e}
Control (T_6)	58 ^{f}	2.00 ^{d}	105 ^{e}	9.8 ^{e}	19 ^{f}	10 ^{e}	0.7 ^{f}
Nawab-21							
BS-1 (T_1)	75 ^{d}	4.75 ^{c}	140 ^{c}	14.8 ^{d}	28 ^{d}	18 ^{c}	1.6 ^{d}
BS-2 (T_2)	79 ^{c}	5.25 ^{bc}	148 ^{b}	16.0 ^{c}	30 ^{c}	20 ^{b}	1.8 ^{c}
Consortium (T_3)	92 ^{a}	6.75 ^{a}	175 ^{a}	20.5 ^{a}	37 ^{a}	25 ^{a}	2.8 ^{a}
Chemical fertilizer (T_4)	85 ^{b}	5.75 ^{b}	152 ^{b}	16.8 ^{b}	32 ^{b}	21 ^{b}	2.0 ^{b}
Non-ZSB isolate (T_5)	70 ^{e}	3.75 ^{d}	128 ^{d}	12.8 ^{e}	25 ^{e}	16 ^{d}	1.3 ^{e}
Control (T_6)	66 ^{f}	3.00 ^{d}	120 ^{e}	11.8 ^{f}	23 ^{f}	14 ^{e}	1.1 ^{f}
SE (n = 4)	0.8	0.228	1.2	0.17	0.4	0.4	0.04
Tukey HSD (0.05)	3.4	0.971	5.3	0.71	1.7	1.7	0.17

Values are means (n = 4); letters as in Table 3.

3.4 Yield components and yield

The consortium produced the longest spikes (12.0 and 13.2 cm), the most spikelets per spike (23.6 and 25.9) and the heaviest grains (1000-grain weight 48.5 and 52.6 g) in Akbar-19 and Nawab-21, respectively (Table 5; Fig. 1). Relative to the control, these values represent increases of 50–60%, 47–62% and 42–50%.

Grain yield showed the largest relative response of any reproductive trait. Consortium inoculation raised yield from 1.5 to 3.8 g $plant^{-1}$ in Akbar-19 (+153%) and from 2.0 to 4.5 g $plant^{-1}$ in Nawab-21 (+125%). These yields exceeded those under chemical fertilization (2.8 and 3.5 g $plant^{-1}$) by 36% and 29%, and those under the better single isolate, BS-2 (2.3 and 3.1 g $plant^{-1}$), by 65% and 45%. The yield gain from the consortium (+2.3 and +2.5 g $plant^{-1}$) was larger than the sum of the gains from BS-1 and BS-2 applied separately (+1.4 and +1.9 g $plant^{-1}$). The significant cultivar × treatment interaction for grain yield arose because absolute treatment differences were somewhat larger in Nawab-21, whereas the relative response to the consortium was larger in Akbar-19; treatment ranking was identical in both cultivars. Biological yield followed grain yield, increasing by 84–89% under the consortium. The non-solubilizing isolate increased grain yield by only 15–20%.

Table 5. Yield components and per-plant yield of wheat cultivars under zinc-solubilizing bacterial treatments.

Treatment	Spike length (cm)	Spikelets spike⁻¹	1000-grain wt (g)	Grain yield (g $plant^{-1}$)	Biological yield (g $plant^{-1}$)
Akbar-19					
BS-1 (T_1)	9.2 ^{d}	17.7 ^{c}	38.5 ^{d}	2.1 ^{d}	15.2 ^{d}
BS-2 (T_2)	9.6 ^{c}	18.7 ^{bc}	40.2 ^{c}	2.3 ^{c}	16.3 ^{c}
Consortium (T_3)	12.0 ^{a}	23.6 ^{a}	48.5 ^{a}	3.8 ^{a}	22.5 ^{a}
Chemical fertilizer (T_4)	10.3 ^{b}	19.7 ^{b}	42.1 ^{b}	2.8 ^{b}	18.1 ^{b}
Non-ZSB isolate (T_5)	8.2 ^{e}	15.7 ^{d}	35.1 ^{e}	1.8 ^{e}	13.2 ^{e}

Treatment	Spike length (cm)	Spikelets spike⁻¹	1000-grain wt (g)	Grain yield (g plant⁻¹)	Biological yield (g plant⁻¹)
Control (T_6)	7.5 ^{f}	14.6 ^{d}	32.4 ^{f}	1.5 ^{f}	11.9 ^{f}
Nawab-21					
BS-1 (T_1)	10.5 ^{d}	19.9 ^{c}	42.5 ^{d}	2.8 ^{d}	18.2 ^{d}
BS-2 (T_2)	11.0 ^{c}	21.7 ^{b}	44.5 ^{c}	3.1 ^{c}	19.8 ^{c}
Consortium (T_3)	13.2 ^{a}	25.9 ^{a}	52.6 ^{a}	4.5 ^{a}	26.3 ^{a}
Chemical fertilizer (T_4)	11.5 ^{b}	22.7 ^{b}	46.4 ^{b}	3.5 ^{b}	21.5 ^{b}
Non-ZSB isolate (T_5)	9.5 ^{e}	18.7 ^{cd}	39.5 ^{e}	2.3 ^{e}	16.0 ^{e}
Control (T_6)	8.8 ^{f}	17.6 ^{d}	37.1 ^{f}	2.0 ^{f}	14.3 ^{f}
SE (n = 4)	0.04	0.28	0.29	0.04	0.25
Tukey HSD (0.05)	0.17	1.20	1.23	0.17	1.06

Values are means (n = 4); letters as in Table 3.

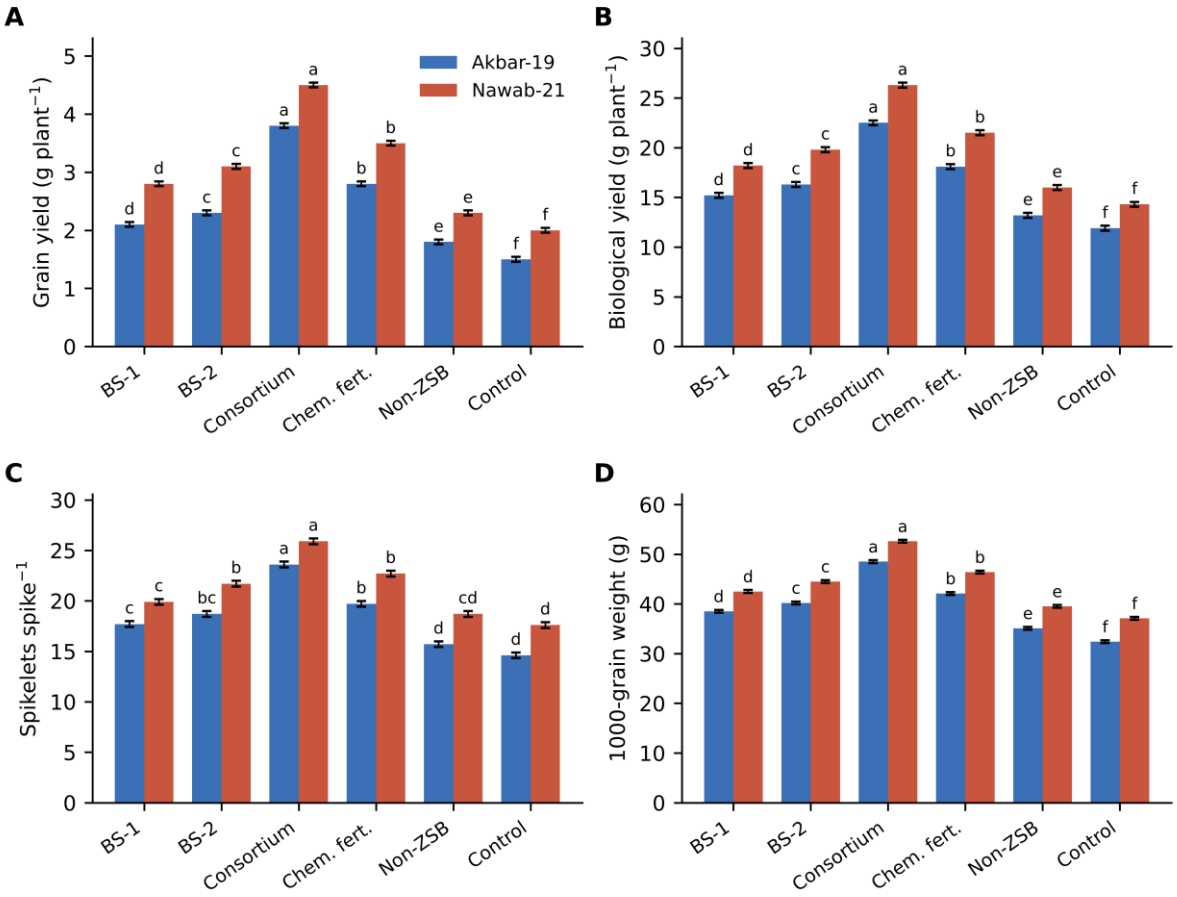


Figure 1. **Grain yield (A), biological yield (B), spikelets per spike (C) and 1000-grain weight (D) of wheat cultivars Akbar-19 and Nawab-21 under single and combined inoculation with zinc-solubilizing bacteria, chemical fertilization, a non-Zn-solubilizing isolate (Non-ZSB) and an uninoculated control. Bars are means ± SE (n = 4); within each cultivar, bars sharing a letter do not differ at $P \leq 0.05$ (Tukey's HSD).**

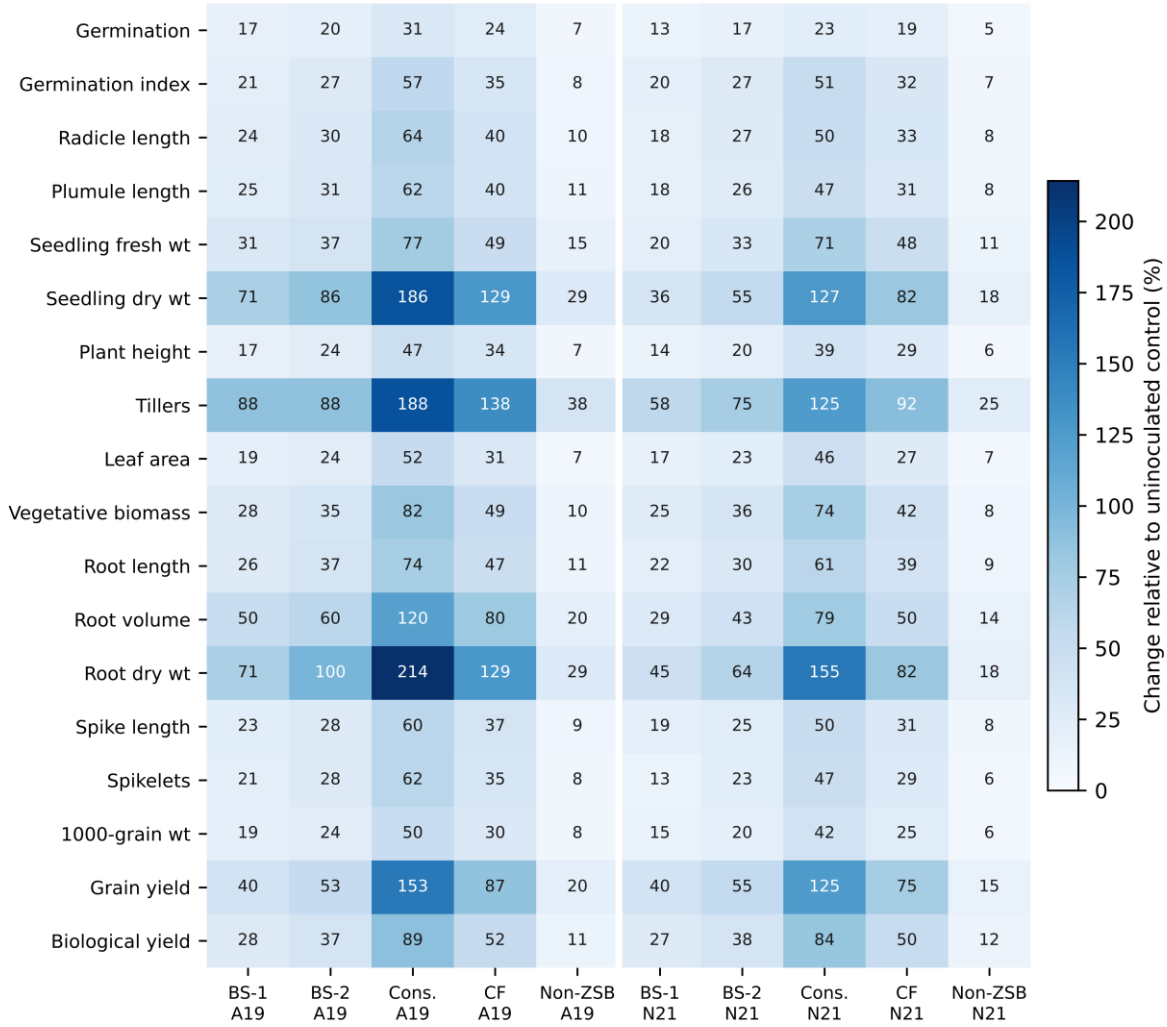


Figure 2. **Relative change (%) in germination, growth, root and yield traits under each treatment compared with the uninoculated control of the same cultivar (A_{19}, Akbar-19; N_{21}, Nawab-21; Cons., consortium; CF, chemical fertilizer; Non-ZSB, non-Zn-solubilizing isolate).**

4. Discussion

4.1 Indigenous ZSB improved establishment and root growth

Inoculation with the two Zn-solubilizing isolates, and especially their consortium, improved every measured trait in both cultivars, from germination to grain yield. The earliest effects were faster and more complete germination and heavier seedlings. Similar improvements in germination and vigour indices of wheat have been reported for Zn-solubilizing rhizobacteria under glasshouse conditions [27], and ZSB-inoculated maize and wheat commonly show larger seedlings and roots [20, 22]. Because these effects appeared in a Petri-dish assay before substantial Zn uptake could occur, they are more plausibly explained by bacterial growth-promoting metabolites in particular auxins, which alter cell elongation and root architecture [18, 19] than by Zn nutrition alone. Testing BS-1 and BS-2 for IAA production, phosphate solubilization and ACC-deaminase activity would clarify which traits are responsible.

Root growth was the most strongly stimulated vegetative component: root dry weight under the consortium was 155–214% greater than in controls and clearly exceeded that under chemical fertilization, whereas shoot height and leaf area responded less. A larger and denser root system increases the soil volume explored and the root–soil interface at which bacterial acidification and chelation can mobilize Zn [17]. Root stimulation can therefore act both as a direct growth effect and as an amplifier of nutrient mobilization. In comparable work, wheat inoculated with ZSB consortia showed the largest increases in root biomass and root Zn content [33,34], and a Bahawalpur field study combining ZSB with Zn-enriched compost reported parallel gains in microbial activity, nutrient availability and yield [31].

4.2 *Consortium versus single* isolates

The consortium outperformed both component isolates for all traits, although each seed lot received a similar total inoculum density. Its grain-yield increment exceeded the sum of the increments produced by BS-1 and BS-2 applied separately, which is consistent with functional complementarity or improved rhizosphere establishment of the mixed inoculum rather than a simple dose effect. Consortia of ZSB have similarly outperformed individual strains for wheat growth and Zn accumulation in controlled studies [33-35], and mixed inoculants can integrate complementary functions such as nutrient solubilization, phytohormone production and stress tolerance [41,42]. However, consortium advantages

are context-dependent: in tomato, a microbial consortium was superior to single strains under the challenging conditions of a desert field site but not under favourable greenhouse conditions [36]. The semi-arid, calcareous conditions at Rahim Yar Khan may similarly favour consortia. Formal tests of synergy (including compatibility assays, rhizosphere population counts of each strain and response-surface designs with varied strain ratios) are needed to confirm an interaction between BS-1 and BS-2.

4.3 Yield formation and comparison with chemical fertilization

Yield gains under the consortium resulted from concurrent increases in tillers, spike length, spikelets per spike and grain weight, suggesting that improved early establishment and root growth were sustained through reproductive development. Field studies with ZSB in wheat have reported comparable increases in yield components, although usually of smaller magnitude [23, 26, 43]. For example, Ali et al. [29] found that indigenous ZSB combined with 8 kg Zn ha^{-1} increased field wheat yield by 38% in calcareous soil, and Khalid et al. [30] reported that ZSB combined with compost and Zn fertilization improved Zn uptake under field conditions. The larger relative responses observed here should be interpreted cautiously, because they are expressed per plant and the control yields were low; plot-scale yields at standard moisture content are needed to judge agronomic relevance.

The consortium also outperformed the chemical-fertilizer treatment for all traits. This comparison should not be interpreted as evidence that the inoculant can replace fertilizer, because the fertilizer treatment was not inoculated and the bacterial treatments did not receive fertilizer. Integrated designs combining inoculation with graded fertilizer and Zn rates have shown that ZSB can increase the efficiency of applied nutrients [29 43-46], and bio-activated Zn formulations have improved both yield and grain Zn of cereals [47 48]. The most practical next step is therefore a factorial trial crossing the consortium with recommended and reduced NPK and Zn rates.

4.4 Specificity of the response and cultivar differences

The non-Zn-solubilizing isolate produced only small improvements over the control (≤ 20% for grain yield), whereas the ZSB isolates and their consortium produced large effects. This contrast argues against a non-specific effect of seed handling, sugar adhesive or bacterial biomass and supports a role for the traits that distinguish the selected isolates — of which Zn solubilization was the selection criterion. Because isolates differ in many traits, however, the comparison does not by itself attribute the response to Zn mobilization; measurements of soil DTPA-Zn and plant-tissue Zn are needed for that attribution.

Nawab-21 outperformed Akbar-19 for every trait, but both cultivars ranked the treatments identically. Grain yield was the only reproductive trait with a significant interaction, reflecting a larger absolute but smaller relative consortium response in Nawab-21. Genotypic variation in responsiveness to ZSB and to Zn fertilization has been documented in wheat [12, 13, 28], and site factors strongly modify the performance of Zn-efficient cultivars [14]. Multi-site testing is therefore required before cultivar-specific recommendations can be made.

4.5 Implications for bio fortification and limitations

This study was conducted within a bio fortification programme, but grain Zn concentration was not measured, so the results demonstrate improved crop performance rather than bio fortification. Higher grain yield may even dilute grain Zn if Zn uptake does not increase proportionally [10]. Several ZSB studies in wheat have shown concurrent increases in yield and grain Zn [23-25 29 43], which makes this a testable expectation for the present consortium. Further limitations are that the results come from a single site and season, that pre-sowing soil properties and plant-available (DTPA-extractable) soil Zn were not analysed, that the isolates have not yet been identified to species, and that the persistence of the inoculated strains in the rhizosphere was not monitored. Drought tolerance was not tested; although bacterial inoculants can alleviate water stress in wheat fields [44], the present experiment was irrigated and did not include a water-deficit treatment. Future work should (i) identify BS-1 and BS-2 by $_{16}$S rRNA gene or genome sequencing and confirm their biosafety; (ii) quantify Zn solubilization and other growth-promoting traits in vitro; (iii) measure soil available Zn and Zn concentration in grain and straw, together with phytate: Zn molar ratios; (iv) monitor strain colonization; and (v) repeat the trial over at least two seasons and several sites using plot-level yields and economic analysis [21 32].

5. Conclusions

Seed inoculation with zinc-solubilizing bacteria isolated from the wheat fields of Rahim Yar Khan improved wheat performance from germination to harvest. *Both indigenous* isolates enhanced germination, seedling vigour, vegetative growth, root development and yield components of Akbar-19 and Nawab-21, but their two-strain consortium was the most effective treatment for all 19 traits in both cultivars. *The consortium raised* germination to 92–96%, more than doubled root dry weight, increased 1000-grain weight by 42–50% and raised grain yield by 125–153% over the control. Its grain yield also exceeded that of the best single isolate by 45–65% and that of recommended chemical fertilization by 29–36%. In contrast, a non-Zn-solubilizing isolate produced only small gains, linking the response to the selected Zn-solubilizing isolates rather than to seed coating as such. Improved early establishment and root growth, probably supported by complementary plant growth-promoting functions of the two strains, provide a plausible basis for the higher yield of the consortium. *The consortium* is therefore a promising candidate for a locally adapted, low-cost bio-inoculant for the wheat–cotton system of southern Punjab. However, these results come from one site and one season, and grain Zn was

not measured. Strain identification and biosafety assessment, measurement of soil and grain Zn, and multi-site, multi-season trials combining the consortium with reduced fertilizer rates are required before it can be recommended to farmers or as a bio fortification input.

Declarations

Author contributions: S. L: Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. *N*. B: Methodology, Validation, Writing – review and editing. M. A. *U*: Conceptualization, Methodology, Resources, Supervision, Project administration, Funding acquisition, Writing – review and editing. All authors read and approved the final manuscript.

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Institutional review board statement: Not applicable. This study did not involve human participants or animals. Field sampling of rhizosphere soil was carried out with the permission of the landowner.

Informed consent statement: Not Applicable.

Data availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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