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Zinc solubilizing *Bacillus* sp. ZM20 and *Bacillus aryabhattai* ZM31 promoted the productivity in Okra *(Abelmoschus esculentus* L.*)*

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ABSTRACT

Zinc solubilizing bacteria (ZSB) improve crop productivity by increasing bioavailability of zinc (Zn). A pot experiment was conducted to evaluate the effectiveness of five promising ZSB strains on the productivity of okra. The experiment was conducted using Completely Randomized Design (CRD) with four replications. Data regarding physiological, growth, and yield parameters were collected and statistically analyzed. Results showed that inoculation of ZSB strains significantly increased these attributes of okra. Inoculation of strain *Bacillus* sp. ZM20 followed by *Bacillus aryabhattai* ZM31 was significantly more effective among the tested ZSB strains. Strain *Bacillus* sp. ZM20 improved relative water contents up to 17%, chlorophyll a and b up to 67 and 71%, respectively, plant height up to 30%, shoot fresh weight up to 19%, shoot dry weight up to 31%, root length up to 79%, root fresh weight up to 58%, root dry weight up to 66%, number of fruits plant^{-1} up to 89%, fruit fresh weight up to 79%, fruit dry weight up to 78%, concentration of N up to 20%, P up to 65%, K up to 20%, and protein contents up to 20% as compared to uninoculated control. It is concluded that inoculation of ZSB strains like *Bacillus* sp. ZM20 and *Bacillus aryabhattai* ZM31 is an effective approach to improve the productivity of okra *(Abelmoschus esculentus L.)*.

Keywords: Okra *(Abelmoschus esculentus L.)*, *Bacillus* strains, Nutrient solubilization, Plant nutrition, Sustainable

INTRODUCTION

Okra *(Abelmoschus esculentus L.)* is widely consumed vegetable crop. It provides high nutritional contents such as carbohydrates, minerals, proteins, calcium, iron and vitamins to human diet (Bawa & Badrie, 2016). In Pakistan, 13.9 thousand hectares area is under okra cultivation with a production of 102.6 thousand tons (Khokhar, 2014). Its production is very low in most developing countries including Pakistan because of its reliance on natural soil fertility.

Low solubility of Zn in soils is an important factor for reducing crop yield and production as it plays important role in metabolism of nucleic acid, cell division, synthesis of proteins and synthesis of indole acetic acid (MacDonald, 2000; Rout & Das, 2009). Zn deficiency occurs in 70% soils of Pakistan due to calcareous nature, low organic matter and high pH and causes crop failure (Bapiri *et al*., 2012). Low Zn solubility in soils is the main cause of Zn deficiency in crops rather than a low total Zn contents (Cakmak, 2008; Alloway, 2009). Its low availability decreases the yield and leads to the inferior quality of crop products and is responsible for its deficiency in humans (Rehman *et al*., 2018).

Zinc deficiency in rhizosphere is being corrected via use of manures and chemical fertilizers. Chemical fertilizers enhance the fertility status of soils and productivity of crops but these also affect the soil chemistry negatively and are very costly (Steinshamn *et al*., 2004). Application of manure fulfills the Zn requirements but depends on the factors like soil physico-chemical properties, temperature, moisture, characteristics of manure, and microbial activity in soil (Alloway, 2009). Researcher reported the increase in crop production and quality of food produced by use of rhizobacteria. They colonize in rhizosphere and increase the plant growth through number of primary and secondary metabolites involved in solubility of phosphorus (P), potassium (K), Zn, iron (Fe), biological nitrogen (N) fixation, production of

Author's Contribution: I.F. Performed all experiments, analyzed data and wrote first draft of manuscript; **M.J., & M.A.** Developed the idea of research, supervised research & edited manuscript.; **M.L.** Helped for the analysis of data**; M.Z.M., S.H., A.H.** Contributed to writing the manuscript**, S.R.K** Helped for the preparation of revision of manuscript suggested by reviewers siderophores, syntheses of phytohormones and control of plant pathogens (Freitas *et al*., 2007; Lugtenberg & Kamilova, 2009; Mumtaz *et al*., 2017).

Most of soils contain significant Zn concentration in unavailable forms which can be converted in to available form by inoculation of Zn solubilizing bacterial (ZSB) strains (Saravanan *et al*., 2004; Bapiri *et al*., 2012; Mumtaz *et al*., 2017, 2018; Khanghahi *et al*., 2018). They dissolve the insoluble Zn compounds via producing organic acids like gluconic acid and 2-keto gluconic acids (Bapiri *et al*., 2012). Inoculation of ZSB in rhizosphere enhances the concentration of Zn and decreases the dependence on synthetic fertilizers. Unwise and indiscriminate use of perilous agricultural chemicals can be reduced by inoculation of ZSB which are good substitute of chemicals for increasing the growth and yield of plants (Vessey, 2003). Therefore, keeping in view the above scenario, the present study was conducted to evaluate the impact of ZSB strains on growth, physiology and productivity of okra.

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MATERIALS AND METHODS

Collection of bacterial strains and preparation of inoculum

Five ZSB strains *viz*. ZM19, ZM20, ZM27, ZM31, and ZM50 were obtained from gene bank of Soil Microbiology and Biotechnology Laboratory, Department of Soil Science, The Islamia University of Bahawalpur. These strains were previously characterized, screened, and evaluated for plant growth promotion by Mumtaz *et al*. (2017, 2018). Among these tested strains, strains ZM20 and ZM31 were identified as *Bacillus* sp. ZM20 and *Bacillus aryabhattai* ZM31 (Mumtaz *et al*., 2017). The bacterial cultures were grown in DF-minimal salt broth amended with 0.1% zinc oxide (ZnO) in shaking incubator (Model SI9R-2, Shellab-USA) for 48 h. After incubation, bacterial cultures were maintained to uniform population (cell count of 10^8 cfu ml^{-1}) and used for seed inoculation.

Seed inoculation and experimental management

Seeds of okra variety *Sabz Pari* was purchased from local seed market of Bahawalpur and sterilized by following method of Khalid *et al*. (2004) and dipped in the respective bacterial culture for 30 mints before seed sowing. Whereas, the control seeds were dipped in broth. Pot experiment was performed at the wire house of Department of Soil Science, The Islamia University of Bahawalpur, Pakistan, located at Lat: 29.40N, Lon: 71.68E and 116 meters elevation above the sea level. Pots were filled with 12 kg sieved loamy soil. Inoculated seeds were sown in pots arranged in Completely Randomized Design (CRD) having four replicates. Recommended doses of N, P and K (50: 25: 25 kg ha^{-1}) were applied in the form of Urea, Diammonium Phosphate (DAP) and Muriate of Potash (MOP), respectively. Full P and K were applied at sowing time while N was applied in three splits doses: first dose at sowing and remaining at 15 days interval. All the recommended agronomic practices were carried out. At physiological maturity, data regarding physiological attributes were recorded while at harvesting; growth and yield parameters were estimated.

Plant analysis

At flowering stage, relative water content (RWC) of top fully developed okra leaf was determined by using formula described by Mayak *et al*. (2004). Chlorophyll a and b contents were also determined spectrophotometically and values were calculated by method of Arnon (1949). Okra fruits were harvested at marketable stage and biometrical observation like number of fruits $plant^{-1}$, fresh and dry weight of fruits were recorded.

For chemical analysis, 100 g of mix okra shoot and leaf were dried in an oven at 65 °C to constant weight and grounded into powder. Plant samples were digested as described by Wolf (1982). The N contents in plant samples were determined by Kjeldal method while P concentration was estimated through adopting procedure of Jackson (1973). The K concentration was determined through flame photometer model BWB-XP (BWB technology Ltd. UK). Values were compared with calibration curve of KCl standard ranging from 0 to 100 ppm and actual concentration was calculated.

The data of various attributes was analyzed for analysis of variance techniques (ANOVA) in accordance with CRD design and means were compared by least significant difference (LSD) test at 5% probability (Steel *et al.*, 2007).

RESULTS

Physiological parameters

Inoculation with ZSB strains significantly increased RWC and chlorophyll ‘a’ and ‘b’ contents (Table I). Inoculation with strain ZM20 reported maximum increase up to 16.8% in RWC as compared to uninoculated control. The strain ZM31 also showed better increase up to 4.2% and was non-significant to strains ZM19, ZM27, and ZM50 but significantly different from uninoculated control. The maximum chlorophyll ‘a’ and ‘b’ contents with an increase up to 66.7 and 70.6%, respectively were observed due to strains ZM20 followed by strain ZM31 that gave 54.5 and 61.8% more chlorophyll a and b contents, respectively, over uninoculated control.

Table I: Effect of zinc solubilizing bacteria on relative water contents, chlorophyll ‘a’ and chlorophyll ‘b’ contents of okra leaves

Treatments	Relative water contents (%)	Chlorophyll ‘a’ (µg/g)	Chlorophyll ‘b’ (µg/g)
Control	68.46 c	0.99 d	1.02 f
ZM19	69.63 bc	1.39 c	1.57 c
ZM20	79.98 a	1.65 a	1.74 a
ZM27	69.71 bc	1.32 c	1.50 d
ZM31	71.48 ab	1.53 b	1.65 b
ZM50	70.65 ab	1.05 d	1.39 e
LSD (p≤0.05)	1.5517	0.0752	0.0582

Means sharing different letters are statistically significant from each other at 5% level of probability (n = 4)

Table II: Effect of zinc solubilizing bacteria on plant height, shoot fresh and dry weight of okra

Treatments	Plant height (cm)	Shoot fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})
Control	80.19 f	125.32 f	75.74 f
ZM19	93.90 c	138.91 c	91.59 c
ZM20	104.03 a	149.26 a	99.29 a
ZM27	90.28 d	134.39 d	85.21 d
ZM31	98.66 b	145.87 b	96.93 b
ZM50	86.53 e	130.44 e	81.17 e
LSD (p≤0.05)	2.5505	2.4762	2.0172

Means sharing different letters are statistically significant from each other at 5% level of probability (n = 4)

Table III: Effect of zinc solubilizing bacteria on root length, root fresh and dry weight of okra

Treatment	Root Length (cm)	Root fresh weight (g plant^{-1})	Root dry weight (g plant^{-1})
Control	44.02 f	59.75 f	29.41 f
ZM19	64.13 c	80.75 c	38.56 c
ZM20	78.77 a	94.50 a	48.83 a
ZM27	61.07 d	76.75 d	35.36 d
ZM31	72.80 b	89.25 b	42.82 b
ZM50	53.80 e	71.50 e	32.16 e
LSD (p≤0.05)	1.7231	1.8856	1.2940

Means sharing different letters are statistically significant from each other at 5% level of probability (n = 4).

Table IV: Effect of zinc solubilizing bacteria on number of fruits plant^{-1}, fresh and dry weight of okra fruit

Treatment	Number of fruits Plant^{-1}	Fruit fresh weight (g)	Fruit dry weight (g)
Control	8.5 e	66.09 f	11.45 e
ZM19	13.6 b	97.05 c	15.09 c
ZM20	16.0 a	118.03 a	20.49 a
ZM27	12.2 c	94.25 d	13.94 d
ZM31	14.8 b	110.11 b	16.99 b
ZM50	10.9 d	87.90 e	13.53 d
LSD (p≤0.05)	0.0264	1.4779	0.9722

Means sharing different letters are statistically significant from each other at 5% level of probability (n = 4)

Table V: Effect of zinc solubilizing bacteria on NPK and protein % age in okra

Treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Protein (%)
Control	2.18 e	0.26 d	1.50 f	13.63 e
ZM19	2.42 c	0.34 c	1.76 c	15.13 c
ZM20	2.62 a	0.43 a	1.80 a	16.37 a
ZM27	2.38 c	0.33 c	1.71 d	14.91 c
ZM31	2.46 b	0.39 b	1.82 b	15.40 b
ZM50	2.31 d	0.27 d	1.63 e	14.48 d
LSD ($p \leq 0.05$)	0.0414	0.0196	0.0264	0.2577

Means sharing different letters are statistically significant from each other at 5% level of probability (n = 4)

Agronomic parameters

Results showed that inoculation of ZSB strains was effective in improving agronomic attributes in terms of plant height, shoot fresh and dry weight, root length, and root fresh and dry weight as compared to uninoculated control (Table II). The maximum increase in plant height, shoot fresh and dry weight was given by strain ZM20 with increase up to 29.7, 19.1, and 31.1%, respectively, followed by ZM30 that improved these attributes up to 23.0, 16.4, and 28.0%, respectively, over uninoculated control.

Significant variation in root growth in terms of root length, root fresh and dry weight was observed in as inoculated as compared to uninoculated control (Table III). Inoculation of ZM20 reported maximum increase up to 78.9, 58.2, and 66.0%, in root length, root fresh and dry weight, respectively, of okra plants as compared to uninoculated control. Inoculation of strain ZM31 were also able to show better root length, root fresh and dry weight with increase up to 65.4, 49.4, and 45.6%, respectively, over uninoculated control.

Yield parameters

Among yield contributing attributes of okra, number of fruits plant^{-1} were significantly promoted due to inoculation with ZSB strains (Table IV). Uninoculated control reported minimum number of fruits plant-1 which were 8.5. Among inoculation treatment, strain ZM20 reported maximum number of fruits plant^{-1} over uninoculated control which were 16, while strain ZM50 gave poor number of fruits plant^{-1} however significantly different from uninoculated control. Data regarding the effect of ZSB strains on okra fruit fresh and dry weight (Table IV) showed that fruit biomass was improved due to inoculation. The maximum fruit fresh and dry weights were observed due to strain ZM20 being 118.0 and 20.5 g plant^{-1}, respectively, followed by the inoculation with ZM31 that showed 110.1 and 16.9 g plant^{-1} of fruit fresh and dry weight, respectively. Minimum fruit fresh and dry weights were observed from uninoculated control.

NPK and protein contents

The improvement in NPK and Protein concentration in straw was observed due to inoculation treatments (Table V). The inoculation of strain ZM20 increased N concentration up to 20.2% as compared to uninoculated control. The maximum increase in P, K, and protein concentration up to 65.0, 20.0, and 20.1%, respectively was also observed by strain ZM20 as compared to uninoculated control.

DISCUSSION

Zinc solubilizing bacteria (ZSB) can promote crop productivity through improving soil fertility. These microbes improved plant health under normal as well as environmental stress conditions and reduced the dependence on hazardous chemicals. In the present study, ZSB strains improved the physiological attributes like RWC, chlorophyll ‘a’ and chlorophyll ‘b’ contents of okra plants. Improvement in RWC might be due to increase in root surface area that enhanced water uptake. These results were supported by Ahmad *et al*. (2011) who described that co-inoculation of rhizobacterial and rhizobial strains improved root length that helped water uptake from depth. Similarly, Egamberdiyeva (2007) and Mumtaz *et al*. (2018) also found that application of rhizobacteria improved root length and root surface area that increased water uptake from far places and resulted in improvement of relative water content. Nayak *et al*. (1986), stated that plants inoculated with PGPR showed increase in chlorophyll contents and photosynthetic rate which led to overall improvement in plant health. Increase in chlorophyll a and b was similar to findings of Sharma *et al*. (2003) who reported the increased in chlorophyll ‘a’ and chlorophyll ‘b’ in rhizobacterial inoculated plants that resulted in increased growth and yield.

The present study showed that inoculation of ZSB strains significantly improved the growth of okra. It could be due to the ability of bacterial strains to create favorable conditions for vegetative growth and to increase shoot and root growth by making nutrients more available to the roots (Adesemoye & Ugoji, 2006). Han *et al.* (2007) studied that soil microbes used as bio-fertilizers play vital functions in decaying organic matter, nutrient cycling and supporting crop growth and health. Richardson (2001) described that rhizobacterial inoculation efficiently increased the root surface area and biomass due to more production of phytohormones by bacterial strains that facilitated more nutrient absorption. Current study also reported the increase in fruit biomass due to ZSB strains which was similar to the findings of Jayapandi & Balakrishnan (1990) who reported the increase in yield component of okra as a result of application of rhizobacterial strains. It is well-documented that biofertilizer enhanced plant growth and yield through making nutrient more available and improving soil health (Iqbal *et al*., 2013).

Inoculation of ZSB strains increased the NPK and protein contents as compared to uninoculated control under current study. The increase in nutrient concentration in plants could be due to their effect on initiation and development of lateral roots and increased root weight. Ahmad *et al*. (2014) reported the increase in root surface area through root proliferation as a result of bacterial strains inoculation which were responsible for the availability of nutrients. The secretion of acids by bacteria and other behavior of soil microbiota affect the equilibrium towards more nutrient solubility and bioavailability to plant roots for absorption (Saravanan *et al*., 2004). Similarly Estrada *et al.* (2013) and Abaid-Ullah *et al*. (2015) evaluated the bacterial strains for secretions of organic acids in response of insoluble nutrient like P, K, and Zn and reported the production of gluconic, oxalic, citric, malic acids, etc. These organic acids have the power to acidified the soil medium and solubilize insoluble compounds. Thus, Zn solubilizing bacterial strains in present work improved the productivity of okra through improving physiology, growth, and yield and increasing the accumulation of nutrients in okra.

CONCLUSION

Inoculation of zinc solubilizing bacterial strains significantly improved the physiological, growth, yield attributes and nutrients concentration in okra. The strains *Bacillus* sp. ZM20 followed by *Bacillus aryabhattai* ZM31 showed more promising results. These strains are well-capable to convert unavailable forms of nutrients into available forms which is unconventional tool to lessen the nutrients deficiency in plant and produce superior quality plant products. These strains could also be better substitute for farmers to lessen the application of chemical fertilizers for sustainable production of crops.

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