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Influence of zinc solubilizing bacterial co-inoculation with zinc oxide supplement on rice plant growth and Zn uptake

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Abstract
The present study was conducted to depict the potential role of zinc solubilizing bacteria namely BMRR126 and BMAR64 on zinc (Zn) biofortification of rice. The short-term pot experiment was performed with both the bacterial isolates, which showed an augmentation in length and weight (both fresh and dry) of the shoot and root of the plant. The highest Zn accomplishment in the shoot (17.67mg/kg) and root (33.43mg/kg) was determined in the treatment (T5) containing bacterium BMRR126 along with zinc oxide. Surprisingly, treatment T5 also augmented the level of available zinc in soil, which was determined to be 2.82mg/kg. All treatments containing zinc solubilizing bacterial inoculants showed better results compared to the control treatment (T1). Results of the current study indicate that zinc solubilizing bacteria can be exploited as potential bioinoculants for Zn biofortification of rice in a sustainable manner.

Keywords: Biofortification, bioinoculants, rice, zinc (Zn), zinc solubilizing bacteria (ZSB)

1. Introduction

Rice is an important crop grown on a huge basis and considered chief feedstock for a large section of the population resides in South Asian nations. The first biofortification approach was successfully performed in rice, which has shown in the form of developing beta-carotene fortified rice with the aim of circumventing vitamin A deficiency (Strobbe *et al*., 2018) [18]. In the current scenario, the prevalence of zinc-associated malnutrition is widespread in several countries, which can be a major risk factor for outbreak of various diseases in humans (Akhtar, 2013) [1]. Since zinc is an essential micronutrient for living beings, it also supports many cellular activities as an important co-factor for numerous enzymes (Roohani *et al*., 2013) [14]. The dearth of this element causes khaira disease in rice. Sometimes the scarcity of zinc in the soil leads to zinc associated malnutrition in plants. Secondly, the insoluble form of zinc in the soil becomes unavailable to plants (Sharma *et al*., 2013) [17]. It is essential that a considerable level of zinc might be reach to edible part of the crop, because crop based foods play an imperative role in delivering zinc to host such as human and animals. A population that relies on crop-based foods may be at risk of zinc related malnutrition if edible parts of plant have low density of zinc. Therefore, it becomes necessary to rectify this issue of zinc malnutrition by adapting the concept of biofortification. The tactics of biofortification deal with micronutrient-associated malnutrition by augmenting particular or all required micronutrients in edible parts of the crop. The biofortification of a crop can be practiced through agronomic methods, plant breeding and biotechnological or genetic engineering approaches (Khan *et al*., 2019; Upadhayay *et al*., 2018; 2019) [8, 26, 25]. However, these approaches are lucrative and arduous, and low-income farmers cannot afford them. Therefore, adopting organic agriculture, which supports the concept of using zinc solubilizing bacteria, can be a fruitful aspect to increase zinc levels in plants (Bhatt and Maheshwari, 2020; Singh *et al*., 2017) [3, 19]. It has been documented that many zinc solubilzing bacteria increase the zinc content in several edible crops and thus provides a novel scenario of microbial-assisted biofortification for plants (Hussain *et al*., 2018) [5]. Using bioinoculants is always an economically feasible method to prop up plant growth and increased yield in a very sustainable manner (Alori and Babalola, 2018; Parveen *et al*., 2018; Singh *et al*., 2011) [2, 12, 20]. Rhizospheric bacteria have numerous plant growth enhancing traits such as phosphate solubilization, production of siderophore, phytohormones, ammonia, EPS and fixation of atmospheric nitrogen

(Joshi *et al*., 2018; Souza *et al*., 2015) [6, 23]. Besides other plant probiotic traits, rhizospheric bacterial isolates are capable to solubilize insoluble zinc of the soil, which may benefit the plant's zinc enrichment.. (Kamran *et al*., 2017) [7]. The solubilization of the zinc in soil is made possible by the secretion of organic acids by bacterial inoculants in the nearby soil (Mumtaz *et al*., 2017) [11]. In the presence of organic acids, the pH of the soil drops to a level that is a favorable prerequisite for the solubilization of zinc and iron (Hussain *et al*., 2018) [5]. In the current study, two zinc solubilizing bacterial isolates (BMRR126 and BMAR64) were used as bioinoculans to observe their effects on rice plants, particularly the better growth and zinc content enhancement in the shoot and root parts of the plant.

Materials and Methods

A short term pot experiment was performed on rice (Pusa Basmati 1) to illustrate the role of zinc solubilizing bacteria under net house during 2018-19 at Department of Microbiology, CBSH, GBPUA&T, Pantnagar, India (29.0247° N, 79.4896° E). Plastic pots (3 kg capacity) were filled with properly sterilized soil (2 kg). The study was carried out in a complete randomized design with three replications. The experimental consisted seven treatments, *viz*., T1 (Control), T2 (Zinc sulfate as Zn supplement @ 25kg/hectare), T3 (Zinc oxide as Zn supplement @ 60kg/hectare), T4 (ZSB-inoculant 'BMRR126'), T5 (ZSB-inoculant 'BMRR126'+ZnO as Zn supplement @ 60kg/hectare), T6 (ZSB-inoculant 'FMAR64'), T7(ZSB-inoculant 'FMAR64'+ZnO as Zn supplement @ 60kg/hectare). The paddy seeds procured from 'BSPC (Breeder Seed Production Center), Pantnagar, Uttarakhand, India' were surface sterilized as per the method described by Singh *et al*., (2013) [21] and Singh and Goel, (2015) [22] with the following steps: a) sterilization of the seeds in a sodium hypochlorite solution (for 3 minutes) followed by washing five times with autoclaved distilled water; b) treating seeds with ethanol (95%) for 1 minute; and c) sterilization of the seeds in 0.1% $HgCl_2$ solution for 3 minutes, followed by washing eight times with autoclaved distilled water. Two zinc solubilizing bacterial cultures namely BMRR126 and FMAR64 were used as bioinoculants. Before sowing, the seeds anticipated for treatments (T4 toT6) were primed with bacterial inoculants through dipping seed in test bacterial suspension for four hours. In the treatments (T1, T2 and T3), seeds were sown without bacterial priming. In each experimental pot, ten healthy seeds were sown. The plant growth determining parameters (such as shoot and root lengths, fresh and dry weight of plant), zinc content in plant, and soil available zinc content were evaluated at 42 days after sowing. The Zn content in the root and shoot part was determined through atomic absorption spectroscopy (AAS) by adapting the acid digestion method of Estefan *et al*., (2013) [4]. Furthermore, the available Zn in the soil samples of each pot treatment was also estimated by DTPA extraction method of Lindsay and Norvell, (1978) [10].

Results and Discussion

Zinc solubilizing bacterial (ZSB) strains showed profound effect on the shoot and root parameters (Table 1). The treatment (T4) containing single bacterium BMRR126 was effective in increasing the length of the shoot (40.20±0.65 cm) in comparison to the control treatment (T1). While, the treatment T6 containing another single bacterium BMAR64 exhibited the highest value of root length (22.10±0.31 cm).

Table 1: The effect of ZSB strains on plant growth parameters (shoot length, root length, wet weight and dry weight) of rice

Treatments	Shoot length (cm)	Root length (cm)	Fresh weight per plant (g)	Dry weight per plant (g)
T1	29.78±0.74	11.50±0.25	0.528±0.026	0.042±0.004
T2	32.95±0.68	12.87±0.45	0.660±0.048	0.053±0.017
T3	31.83±1.36	12.20±0.15	0.537±0.050	0.046±0.009
T4	40.20±0.65	19.93±0.38	0.835±0.040	0.092±0.008
T5	37.08±0.50	21.17±0.44	0.826±0.014	0.077±0.005
T6	38.20±0.88	22.10±0.31	1.138±0.013	0.103±0.007
T7	36.20±0.67	21.43±0.23	0.814±0.062	0.065±0.001

The data are average of triplicate experimental value; '±' indicates standard deviation

The augmentation of shoot and root length in response to bacterial inoculants depict plant growth supporting behavior as illustrated in studies performed by Ramesh *et al*., (2014) [13]. The plant growth promoting traits of bacteria such as phosphate solubilization and auxin production directly influence plant growth. Treatment T6 having single zinc solubilizing strain FMAR64 showed maximum fresh weight (1.138±0.013 g) and dry weight (0.103±0.007g) of rice. The increased zinc content in different parts of plants after microbial inoculation illustrates the concept of 'microbial assisted biofortification'. In the case of shoot and root part of plant seedlings, the increased zinc content was depicted as 17.67mg/kg for the shoot (Figure. 1) and 33.43mg/kg for the root (Figure. 2) under the response of treatment T5 containing ZSB inoculant 'BMRR126' and recommended dose of zinc oxide. However, all the treatments exerted an increased zinc level both in the shoot and root compared to the control treatment (T1). Similarly, the same treatment (T5) was fabulous, showing remarkable results in terms of an increased amount of available zinc (2.82mg/kg) in the soil when compared to other treatments (Figure. 3). More zinc accumulation in the root part might be due to the better activity and appreciable accessibility of solubilized form of zinc in soil. A related study was also done by Sharma *et al*., (2014) [18] who illustrated an increment of Zn amount in the root and shoot of the plant upon treatment of bacterial inoculants having plant growth enhancing traits. Moreover, the current finding is also supported by study of Krthika and Baachandar, (2016) [9] where the inoculation of *Enterobacter cloacae* (strain ZSB14) with ZnO augmented the Zn level in root and shoot part of rice seedling then the treatment encompassing either single ZnO or single bacterial inoculant. The increased available Zn content in the soil might be due to the activity of bacterial inoculants as various organic acid producing bacteria aids in declining the rhizospheric soil pH (Krthika and Baachandar, 2016; Ramesh *et al*., 2014; Shakeel *et al*., 2015) [9, 13, 16]. Microorganisms such as *Bacillus* and *Pseudomonas* were found to produce organic acids that eventually resulted in the decline of soil pH, and ensure adequate Zn accessibility to plants (Saravannan *et al*. 2004) [15].

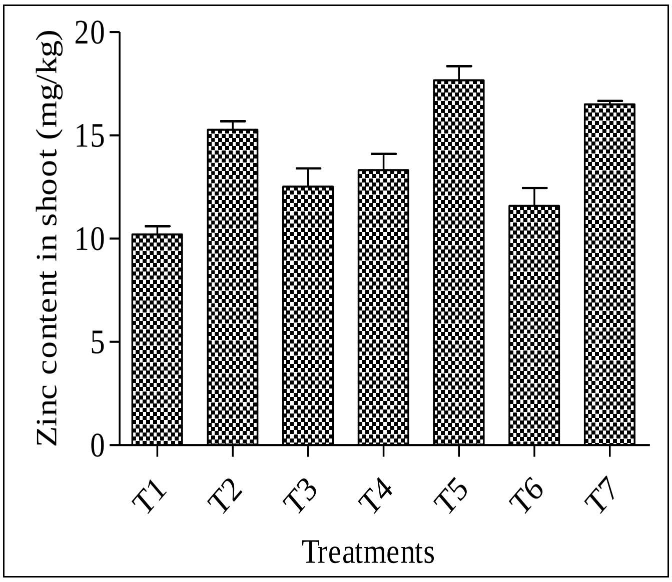

Fig 1: Response of ZSB strain on Zn content of shoot of the plant

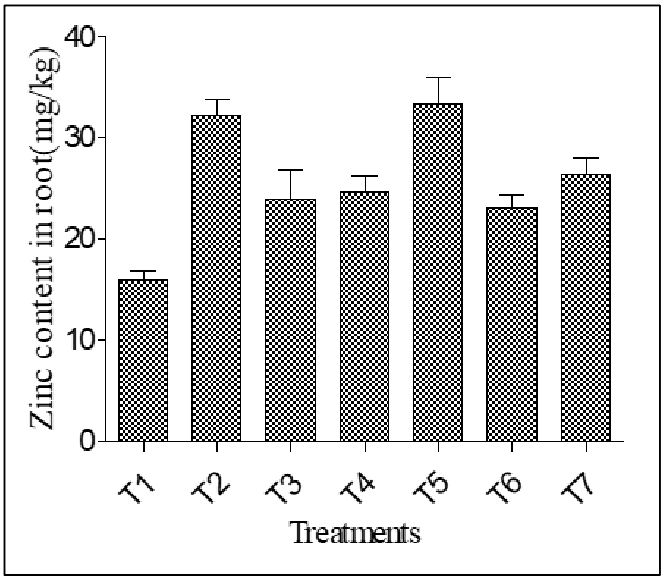

Fig 2: Response of ZSB strain on Zn content of root of the plant

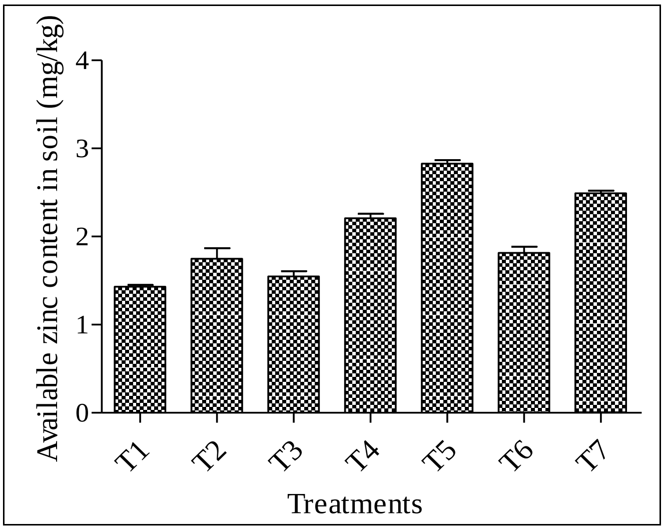

Fig 3: Response of ZSB strain on available Zn content of the soil

Conclusion
The present study illustrated the contribution of zinc solubilizing bacteria on the growth of rice plant. Bacterial strains, namely BMRR126 and FMAR64 were determined to be competent plant probiotic strains because they exhibited increased plant growth in terms of shoot and root length and augmented rice plant biomass. In addition, the effect of bacterial strain BMRR126 with the ZnO improved the Zn contents in shoot and root parts of the plant and illustrated its potential for solving the purpose of Zn biofortification in rice. The outcomes of the study advocate the use of zinc solubilizing bacterial strains in the form of biofertilizers to escalate the uptake of zinc and available zinc both in edible parts of plants and in the soil, repectively.

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