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Rhizobacterial Inoculation to Improve Wheat Growth and Soil Quality in a Saline Environment

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ABSTRACT

Objective: *To elucidate the effects of PGPR inoculation on the growth and productivity of wheat (cultivar AZRC-84) cultivated under saline conditions.* ***Methods:*** *Four salt-resistant PGPR strains were isolated from the wheat rhizosphere at the Arid Zone Research Center (AZRC) DI Khan. A pot experiment, involving these PGPR strains, was conducted on wheat grown in saline soil with an electrical conductivity (ECe) of 7.3 dS m-1. Plant biomass and various soil parameters were analyzed 25 days post-germination.* ***Results:*** *Compared to controls, inoculation with PGPR strains significantly improved the wheat's dry biomass, especially with MB3 Pseudomonas aeruginosa, MB2 Enterobacter mori, and MB4 Enterobacter asburiae treatments. In terms of microbial population under saline conditions, the maximum bacterial populations were observed in MB3 Pseudomonas aeruginosa and MB4 Enterobacter asburiae treatments. Soil analyses post-PGPR inoculation revealed an increase in organic carbon and water-holding capacity. Concurrently, there was a reduction in ECe, pH, and sodium content, whereas the soil's bulk density remained unaltered.* ***Conclusion:*** *PGPR inoculation demonstrates potential in enhancing wheat growth in saline environments by not only promoting plant growth but also ameliorating certain detrimental soil parameters. The findings hold promise for improving crop yields in salt-affected areas through bio-inoculation strategies.*

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INTRODUCTION

About 20% of irrigated agricultural land suffers unfavorable effects from salinity [1]. Salt management issues can cause sodicity in clay soils. When negatively charged clay encounters sodium (Na), the clay expands and disperses. Saline soils have poor plant nutrition and high levels of osmotic stress, have a major detrimental effect on plant growth. Salt stress affects all a plant's key metabolic processes, including development, photosynthesis, protein synthesis, and lipid metabolism [2]. Proline may aid in osmotic regulation, guard macromolecules from desiccation, and serve as a vital part of the body's antioxidative defense mechanism in the pentose phosphate pathway [3]. When exposed to salinity, plants of the chickpea (*Cicer arietinum L.*) species exhibited heightened Na/K fraction and lower P assimilation in shoot [4]. But inoculating plants with PGPR can benefit plants to grow better under osmotic stress [5]. Utilizing PGPR bio-inoculants, i.e., Agrobacterium, Pseudomonas, Azospirillum, and numerous other bacterial species, is an ecologically responsive, and economically feasible method of recovering salinity-stricken land and improving biomass output [6]. As PGPR colonize plant roots, its use can be beneficial in the creation of strategies to elevate the growth of wheat in salty conditions [7]. Inducing PGPR chemotaxis on root surfaces by root exudates i.e., carbs and amino acids enhances the possibility that bacteria will reach the plant roots [8]. There have been reports of higher agronomic yields because of PGPR due to the generation of growth-stimulating plant hormones i.e., gibberellic acid (GA), indole-3-acetic acid (IAA), ethylene, zeatin, abscisic acid and phosphorus solubilization [9]. Salinity significantly reduces the yield of wheat, which on moderately salinized soils results in a loss of about 65 percent of the crop [10]. Although there is very little information available regarding the role of PGPR in wheat under salinity, it has been observed that using PGPR inoculum for cereal growth can decrease salt stress [11]. This research investigated the effects of PGPR application on the development and productivity of wheat in a saline environment.

MATERIALS AND METHODS

PGPR Collection

The current research utilized four salt resistant PGPR, which were isolated from the wheat rhizosphere at Arid Zone Research Center (AZRC) DI Khan. These PGPR were found to have a w/v concentration of 6% sodium chloride. These strains have growth-promoting properties i.e., IAA, P solubilization, GA, proline, siderophores, reducing sugars (RS), and total solvable sugar synthesis at 6% sodium chloride for plants [5].

Testing and analyzing cannabis

A pot experiment with the salt-tolerant wheat cultivar AZRC-84 that was inoculated with PGPRs (MB_1 *Pseudomonas putida*, MB_2 *Enterobacter mori*, MB_3 *Pseudomonas aeruginosa* and MB_4 *Enterobacter asburiae*) was carried out at AZRC DI Khan. The salinity of the soil was measured at EC_e = 7.3 dS m^{-1}. 25 days after the seedlings emerged, the plants were dug up and observations were conducted on the overall as well as the shoot and the roots (dry biomass). According to the procedures developed by Kalra and Maynard, the bulk density, organic carbon, electrical conductivity (ECe), and water-holding capacity of the pre-treated (saline soil prior to bacterial inoculum) and post-treated (rhizosphere soil following bacterial inoculum 25 days after sowing) soil from wheat grown in containers were analyzed [12]. Bacterial populations in the soil around the rhizosphere were assessed for the number of colony-forming units (CFU) that they produced on a NA medium (Upadhyay et al. [5] and Upadhyay et al. [7] methods were used to calculate the salt content of the soil.

Statistical analysis

Statistix version 8.1 was utilized throughout all of the analyses of variation. When conducting multiple range studies, the LSD test was utilized to identify significant differences between the several sets of data. When $p<0.05$, the findings were deemed to be significant.

RESULTS

Wheat plants' response to PGPRs in salty environments

Controls consisted of non-inoculated treatments that were either subjected to or not subjected to NaCl stress. Extreme biomass was attained with inoculation of isolates MB_3 *Pseudomonas aeruginosa*, MB_2 *Enterobacter mori* and MB_4 *Enterobacter asburiae*. Highest root biomass was attained following inoculation with MB_3 *Pseudomonas aeruginosa*. Inoculation with isolates MB_3 *Pseudomonas aeruginosa*, MB_2 *Enterobacter mori* and MB_4 *Enterobacter asburiae* significantly improved total dry biomass (Table 1).

Effect of PGPRs on microbial population under saline conditions

The rhizosphere possessed the most bacterial populations, and all of the PGPR strains showed rhizo-adaptation in wheat (i.e., the optimal Cfu population; see Table 2). The greatest was observed in MB_3 *Pseudomonas aeruginosa* and MB_4 *Enterobacter asburiae*.

Effect of PGPRs on soil parameters under saline conditions

After PGPR was inoculated into the soil, there was a greater increase in organic carbon and water-holding capacity in comparison to the control. On the other hand, the ECe, pH, and sodium content of the soil all decreased, but the bulk density of the soil remained the same (Table 3).

Table 1: The influence of PGPR on the growth characteristics of wheat (AZRC-84) grown in salty environments

Treatments	**Shoot**		**Root**		**Total**	
	Fresh biomass (g)	**Dry biomass (g)**	**Fresh biomass (g)**	**Dry biomass (g)**	**Fresh biomass (g)**	**Dry biomass (g)**
Uninoculated (Normal Soil)	0.69 c	0.17 b	0.30 b	0.08 b	0.99 c	0.25 c
Uninoculated (Saline Soil)	0.42 e	0.11 d	0.18 d	0.05 c	0.60 e	0.16 e
MB_1 *Pseudomonas putida*	0.57 d	0.14 c	0.25 c	0.07 bc	0.82 d	0.21 d
MB_2 *Enterobacter mori*	0.73 b	0.18 b	0.32 b	0.09 ab	1.05 b	0.27 b
MB_3 *Pseudomonas aeruginosa*	0.87 a	0.22 a	0.38 a	0.10 a	1.25 a	0.32 a
MB_4 *Enterobacter asburiae*	0.71 b	0.18 b	0.31 b	0.08 b	1.02 bc	0.26 bc

Table 2: PGPR's impact on the bacterial population under salty environments

Treatments	**BP (cfu g^{-1} soil)**
Uninoculated (Normal Soil)	6×10^4
Uninoculated (Saline Soil)	3×10^2
MB_1 *Pseudomonas putida*	7×10^5
MB_2 *Enterobacter mori*	4×10^6
MB_3 *Pseudomonas aeruginosa*	7×10^7
MB_4 *Enterobacter asburiae*	6×10^7

Table 3: The influence of PGPR on soil characteristics in salty environments

Treatments	**EC_e (dS m^{-1})**	**pH**	**Na (ppm)**	**BD (Mg m^{-3})**	**Organic Carbon (%)**	**WHC (%)**
Uninoculated (Normal Soil)	2.69 d	8.1 ab	13 c	1.18 d	0.96 c	25.2 b
Uninoculated (Saline Soil)	6.8 a	8.3 a	38 a	1.38 a	0.65 e	17.8 d
MB_1 *Pseudomonas putida*	3.94 b	8.2 ab	18 b	1.33 b	0.81 d	21.3 c

MB_2 *Enterobacter mori*	3.05 c	7.9 c	14 c	1.31 bc	0.97 bc	27.5 a
MB_3 *Pseudomonas aeruginosa*	2.98 cd	7.9 c	13 c	1.28 c	1.15 a	27.9 a
MB_4 *Enterobacter asburiae*	3.17 c	8.0 b	13 c	1.30 bc	1.03 b	26.8 a

DISCUSSION

Wheat has a tolerance level that falls in between moderate and high for salinity, and there have been reports of species differences in salinity tolerance [13]. Free-living bacteria that either directly or indirectly promote plant growth are referred to as plant growth-promoting bacteria [14]. In trials conducted in greenhouses, all four PGPR utilized had a substantial impact on the development of wheat and the health of the soil in pot experiments conducted inside a saline environment; in other words, all four PGPR stimulated growth [5]. After PGPR inoculation, there may be an improvement in nutrition in the saline environment, which may account for the rise in biomass. The detrimental effects of salt on the growth of tomato, pepper, canola, cotton, and wheat have been demonstrated to be partially mitigated by the use of certain PGPR in prior studies [15]. Rhizo-adaptation or a rhizosphere effect can be demonstrated by the fact that the population of PGPR grew over time after sowing in the rhizosphere soil of PGPR-treated plants (Table 2). Hiltner, was the first person to describe the rhizosphere effect, which can be defined as the attraction of microorganisms to nutrients that are released from plant roots, leading to an increase in the number and activity of microorganisms in the area surrounding plant roots [16]. However, in addition to providing an environment rich in carbon, plant roots also initiate crosstalk with soil microbes. This causes plant roots to produce signals that are recognized by soil microbes, which then cause soil microbes to send signals that initiate colonization [17]. These bacterial populations will colonize the rhizosphere and interact with one another by a variety of mechanisms, including root exudates and chemotaxis, symbiosis, quorum- sensing, and others [18]. In addition to having an effect on plant growth, PGPR also improves the health of salty soil. In PGPR-treated soil, organic C and water-holding capacity increased, whereas ECe, sodium content, and pH fell. This was in comparison to the controls, which were grown in soil that had not been treated. The production of organic acids with low molecular weight by bacteria capable of phosphate solubilization may contribute to a decrease in pH.[19]. The solubilization and mineralization processes can co-exist in certain bacterial strains, according to Tao et al. [20]. This contributes to an improvement in soil health by keeping the pH close to neutral. The formation of exopolysaccharides by PGPR strains also assists in binding cations, including sodium, and as a result, it may reduce the amount of sodium that is available for plant absorption and contribute to the alleviation of salt stress [7].

CONCLUSION

Based on the findings from this investigation, we can ascertain several key insights. Despite the evident benefits of PGPR inoculants, there remains a significant gap in our comprehensive understanding of their exact mechanisms and modes of action. In environments burdened by salinity, the introduction of PGPR appears to be a promising strategy to enhance soil health and vitality. Particularly noteworthy among the studied rhizobacteria is Pseudomonas aeruginosa, which stands out for its robust salt-resistance and potential to substantially augment wheat growth under saline conditions. Moreover, PGPR serve as not only eco-friendly alternatives but also effective countermeasures against the detrimental impacts of excessive fertilizer usage in agricultural practices. Consequently, they play a pivotal role in ensuring a harmonious balance between optimal crop yield and soil preservation, furthering the cause of sustainable agricultural endeavors.

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