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Salinity amelioration and plant growth promotion by *Paenibacillus castaneae* in tomato

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ABSTRACT

The present study stated that salt-tolerant plant-associated *Paenibacillus castaneae*. enhance plant tolerance to salinity. The treatment using *P. castaneae* strain presented noticeable but varying effects on plant growth under and often displayed a significant increase in germination percentage, root and shoot length, and other growth parameters of tomatoes compared to those in the non-inoculated control.

The physiological responses *viz*., proline, relative water content and chloprphyll showed were increased as compared with the control treatment better growth promotion significantly by the application of *P.castaneae* in the saline environment. The strategy against oxidative damage by increasing antioxidant enzyme activities under high salinity stress. These results suggest that salt-induced oxidative stress in tomatoes is generally counteracted by enzymatic defense systems stimulated under harsh conditions. The field experiment was conducted with the laboratory results obtained in this *P. Castaneda* along with Azospirillum and phosphobacteria was studied on tomato cultivars under field conditions. The field study results confirmed that 75% recommended N + 75% recommended P + *Azospirillum* + *Phosphobacteria* + *Paenibacillus* strain exhibited grateful results in improving tomato plant growth and yield under salinity stress.

Keywords- *Tomato, Salt stress, Paenibacillus, yield character, antioxidants*

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Introduction

Abiotic stressors such as salt, drought, and temperature have all had a negative impact on agriculture. These are important impediments to global sustainable agriculture [1].The major abiotic stress conditions of concern are light, temperature, drought, salinity, soil, air, and water contaminants. Salinity is a key abiotic stress and harsh environmental element that has a negative impact on crop productivity and quality. Salinity (salt stress) affects approximately 20% of the total cultivated area and 33% of irrigated agricultural lands. Agricultural production is greatly reduced on soils with high salt concentrations due to poor plant nutrition, osmotic imbalance, and drought stress [2]. Salinity stress induced by NaCl is one of the most prevalent abiotic stresses that impact plant productivity through morphological (e.g., decrease in leaf number and fruit output) and metabolic disorders (e.g., imbalance in stomatal conductance, poor photosynthetic activity) [3].

In recent years lot of research has been used to alleviate the problem of soil salinity and acidity. Alternative approaches for reclaiming salt-affected soils include phytoremediation and bioremediation. Plant growth-promoting rhizobacteria (PGPR) are a diverse group of bacteria renowned for their beneficial properties [3]. The acquisition of nutrients, the solubilization of phosphorus (P), the production of siderophores, the fixation of atmospheric nitrogen (N), the production of hydrocyanic acid (HCN), the regulation of plant hormones, and the defense against biotic pathogens are just a few of the mechanisms used by PGPR to promote plant growth. Plant development is promoted by N_2 fixation, P availability, and hormonal response; other systems indirectly support plant growth [4].

The most common bacteria that tolerate elevated concentrations of NaCl (1-15%) are *Ochrobactrum intermedium, Bacillus subtilis, Pseudomonas fluorescence, Kocuria rhizophila, Bacillus amyloliquefaciens, Bacillus firmus, Azotobacter chrooccocum, P. stutzeri, Azospirillum brasiliense, A.lipoferum, P. putida, Curtobacterium flaccumfaciens, Arthrobacter sp.*, and *Paenibacillus.* These strains were discovered to promote plant growth and sodium chloride tolerance in maize, wheat, barley, rice, soybean, sunflower, and tomato [5,6].

The current work focuses on evaluating *Paenibacillus castaneae* for plant growth-promoting properties in saline soil conditions under *in vitro* and field conditions in tomatoes.

Materials and methods

Microorganisms and Growth Conditions

Paenibacillus castaneae was obtained from, the Culture collection centre of the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Growth analysis *Paenibacillus castaneae* in salt stress

The salt stress tolerance potential of *P. castaneae* was evaluated. The Luria Bertani-broth was made and supplemented with various salinity stress concentrations

(0, 100, 200, and 300 mM NaCl). To ascertain the tolerance capability, the bacterium *P. Castaneae* was inoculated in LB-broth and cultured for 8 days at 150 rpm and 32 °C in a shaking incubator. To assess how well each strain of bacteria could withstand salinity stress, the optical density of each strain was measured at 600 nm every day for seven days. A salt-tolerant bacterial strain was regarded as one with an optical density (OD) of 0.5 in the face of salinity stress. In LB broth and media, the *P. castaneae* proliferated rapidly and exhibited tolerance to salinity stress up to 300 mM NaCl.

In vitro evaluation of *Paenibacillus castaneae* under induced salt stress

A loopful bacterial culture that was 24 hours old was maintained at 10^8 CFU mL/L and aseptically dissolved in phosphate buffer saline (pH 7.4). The cell pellet was re-suspended in a 1% phosphate buffer, which had undergone sterilization. In order to surface-sterilize tomato seeds (PKM 1), 1.0% NaOCl was applied for 1 minute, followed by 70% ethanol for 3 minutes, and finally three rinses with sterile distilled water. The tomato seeds were immersed in a bacterial suspension for priming. The primed seeds were incubated for 24 hours at 28° C. After incubation, seeds were planted in sterile soil in earthen pots [7].

There were four treatment groups: (1) the control group, (2) Soil with 200 Mm (NaCl) group, (3) *Paenibacillu scastaneae* inoculation group, and (4) *Paenibacillus castaneae* + 200Mm (NaCl). Three replications of each treatment were present under controlled circumstances throughout the whole experiment, which used a randomized block design. In each of the treatments, a salt treatment was administered seven days after germination by irrigation with 50 mM NaCl for four days. At 21 days, seedlings were taken and their growth characteristics were assessed.

Physiological Responses of Plants to Salt Stress

The Bates et al. method was used to measure the proline content. At 520 nm, the toluene that included a chromophore was measured ([8]. From a standard curve, the amount of proline was calculated in g/g fresh weight (FW). Chlorophyll estimation was performed by the method of Moran and Porath [9]. The relative water content (RWC) was measured in plant leaves according to the protocol of Sade et al. [10]

Antioxidant Enzyme Activity Assays

Antioxidant enzyme activity was observed in tomato leaf tissues. The extraction of the crude protein followed the procedure described by Qureshi et al [11]. One gram of tissue was homogenized in 4 mL of a 100 mM potassium phosphate buffer (pH 7.4) that also contained 2% polyvinyl pyrrolidone (PVP), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1 mM ethylenediaminetetraacetate (EDTA). The supernatant from the centrifugation of the crude extract at 15,000 g for 20 min at 4 °C was utilized as an enzyme extract.

Determination of peroxidase (POD) enzyme activity was performed as per the method of Kumari et al. [12] . Polyphenol oxidase (PPO) activity was quantified as per the method of Weisany et al. [13] , and activity was expressed in U/mg protein.

Field experiment

The field experiment was conducted at Horticultural College and Research Institute for Women at Trichy district of Tamil Nadu during the Rabi season of 2022-23. The experiment was designed with six treatments and four replications in a randomized block design. Standard cultural practices were followed as recommended by Tamil Nadu Agricultural University, Coimbatore, Tamilnadu. **T_1**– Control, **T_2** – 75% recommended N + 75% recommended P, **T_3** – 100% N, P and K, **T_4** - 75% recommended N + 75% recommended P + *Azospirillum* + *Phosphobacteria* (Seed treatment + Soil application), **T_5** - 75% recommended N + 75% recommended P + *Paenibacillus* strain (Seed treatment + Soil application), **T_6** – 75% recommended N + 75% recommended P + *Azospirillum* + *Phosphobacteria* + *Paenibacillus* strain.

Observations

The following observations *viz.,* plant growth parameters and yield parameters were recorded .

Statistical analysis

The results of the experiments were subjected to statistical scrutiny as per the methods detailed by Panse and Sukhatme [14]. All the treatments were compared at $p \leq 0.01$ and 0.05 levels of significance using the Critical Difference (CD) test which was performed by Excel-2000. The Analysis of Variance (ANOVA), Standard Error (SE), and Critical Difference (CD) for dependent parameters were tabulated and the level of significance was reported.

Result and discussion

Growth curve analysis of *P.castaneae* (VPB1)

It was investigated that *P.castaneae* can endure salinity stress and showed healthy growth in salt-amended LB media. *Paenibacillus castaneae* can resist NaCl concentrations of up to 3 M (Figure 1). As the salinity increased, the bacterial population continued to decline in number. Hence, salinity stress and bacterial growth showed an inverse relation as compared to the control which is similar to that in a previous report of Ali et al. [15].

Effect of *P.castaneae* on Plant Growth Parameters Under Salt Stress

Plant growth characteristics were significantly impacted negatively by salt stress. In comparison to the control condition, salt stress resulted in a reduction in all growth metrics. *P.castaneae,* however, was able to protect plants from serious harm brought on by salt toxicity. In comparison to T_2 treatment, plants inoculated with *P.castaneae* (T_3) showed considerably longer shoot and root lengths (15.36 cm and 7.6 cm). Similar to this, during salt stress, bacterial inoculation dramatically increased plant biomass compared to un-inoculated plants. Under salt stress, plants treated with T_4 had a germination percentage and vigour index than plants treated with T_2, the uninoculated control (Table 1). Both treatments (T_1 and T_3) displayed comparable trends in plant growth parameters under non-salt environments.

Additionally, *P.castaneae* demonstrated abilities to boost plant development, suggesting the possibility that the *Paenibacillus castaneae* isolate may support plant growth in nutrient-restricted environments. According to various studies *Sphingobacterium* spp. exhibit advantageous PGP features with the capacity to promote plant growth under various stress circumstances [16-19] . Additionally, it was discovered that the isolate BHU-AV3 could withstand salt stress up to 4% NaCl concentration [7].

Effect of *Paenibacillus castaneae* on physiological and antioxidant enzymes response to salt stress

Under salt stress, a decrese in chlorophyll concentration was seen. However, compared to uninoculated plants (T_2), *P. Castaneae* (VPB1)inoculated plants (T_3) showed a much higher chlorophyll content (80.12 g/g). Under saltwater circumstances, the relative water content of tomato plants was considerably decreased. Nevertheless, compared to salt treatment plants, microbe inoculated plants accumulated more water content (89%) (Table 2).

Under salt stress, the leaves of *P. Castaneae* (VPB1)-inoculated plants collected more proline than the non-inoculated plants, suggesting that proline plays a role in maintaining the osmotic equilibrium within the root [19,20]. Furthermore, more proline shields membrane enzymes and proteins from oxidative bursts [21]

A comparative study of antioxidant enzyme activities was performed in leaf tissues with different treatments. Salt stress generally stimulates the antioxidant system throughout the plant. *P. castaneae* strain-inoculated leaves (T_3) showed higher POD , SOD and PPO as compared to T_2-treated plant roots. In leaf tissue, un-inoculated plants. (T_2) displayed significant increases in all enzymatic activities compared to T_4 plants. Under non-salt conditions, both treatments (T_1 and T_3) maintained the same level of POD, SOD, and PPO activities.

Field evaluation of *P.castaneae* in tomato

The influence of biofertilizers on plant growth parameters *viz.*, plant height, 50% flowering, No of branches, was recorded at 30 and 60 DAS under saline soil. The yield parameters like number of leaves (57 Nos), plant height (72 cm), days to 50 % flowering (34 days) and first flowering (30 days), and no of branches (10 nos) were highest in 75% recommended N + 75% recommended P + *Azospirillum* + *Phosphobacteria* + *Paenibacillus* strain followed by other treatments (Table 3 and 4). According to Kamanga et al. (22), salinity adaptation lessens the detrimental effects of salt stress, whereas Meza et al. (23) found that moderate salt stress increases tomato fruit quality without affecting yield. By enhancing antioxidant capacity, ion homeostasis, and PA metabolism, lowering ABA and ET levels, and suppressing transpiration, elevated CO_2 provides tomato tolerance to progressively greater soil salinity and secondary soil salinization [24] .

Conclusion

The *Paenibacillus castaneae* (VPB1)- enhances tomato plant growth and yield in the presence of 200 mM NaCl, as well as alleviates the harmful impact caused by salt stress under pot culture conditions. The tomato plant inoculated with bacteria showed more severe changes in accumulating, proline, and antioxidant enzymatic activities compared to the control. Enhanced activities of these parameters in plants resulted in a decrease in oxidative stress in tomato plants, concerning ROS content. The field study results confirmed that 75% recommended N + 75% recommended P + *Azospirillum* + *Phosphobacteria* + *Paenibacillus* strain exhibited grateful results in improving tomato plant growth and yield under salinity stress.

Table 1.Effect of P. castaneae (VPB1) inoculation on tomato plant growth parameters under salt stress conditions

S.No	Treatment Details	Root length (cm)	Shoot length (cm)	Plant biomass (g)	Germination %	Vigour index
1	Control	6.8 (±0.10)^{b}	16.47 (±0.21)^{a}	0.31(±0.003)^{b}	85(±1.12)^{c}	594(±7.88)^{c}
2	Soil with 200 Mm	4.6 (±0.02)^{d}	8.4 (±0.66)^{d}	0.17(±0.002)^{d}	72(±0.90)^{d}	609(±4.76)^{c}
3	*Paenibacillus castaneae*	7.6 (±0.02)^{a}	15.34 (±0.03)^{b}	0.34(±0.002)^{a}	98(±1.16)^{a}	1510(±24.14)^{a}
4	*Paenibacillus castaneae* + 200 mM	5.4 (±0.02)^{c}	12.28 (±0.03)^{c}	0.21(±0.002)^{c}	90(±0.06)^{b}	1110(±15.86)^{b}
	CV	1.86	1.72	1.87	2.15	3.17
	SED	8.02	0.16	3.39	1.32	21.44

Table 2. Effect of P. castaneae (VPB1) on physiological and antioxidant enzymes response to salt stress

S.No	Treatment Details	Physiological response			Antioxidant enzymes		
		Proline (µg/g FW)	Chlorophyll (µg/g FW)	RWC (%)	Catalase µ mol of cinnamic acid/min/g of leaf tissue	Peroxidise absorbance /min/g of leaf tissue	Polyphenol oxidase change in absorbance /min/g of leaf tissue
1	Control	42 (±0.07)^{c}	78.5 (±0.05)^{a}	90.12 (±0.21)^{a}	3.75 (±0.06)^{c}	0.95 (±0.005)^{c}	1.12 (±0.02)^{c}
2	Soil with 200 Mm	105 (±1.71)^{a}	51.34 (±0.35)^{c}	40.65 (±0.53)^{c}	1.18 (±0.02)^{d}	0.65 (±0.008)^{d}	0.78 (±0.01)^{d}
3	*Paenibacilluscastaneae*	38 (±0.13)^{d}	80.12 (±0.84)^{a}	89.34 (±1.15)^{a}	7.21 (±0.09)^{a}	1.92 (±0.003)^{a}	1.85 (±0.02)^{a}
4	*Paenibacilluscastaneae*+ 200 mM	87 (±1.12)^{b}	74.35 (±1.19)^{b}	63.47 (±0.43)^{b}	6.25 (±0.09)^{b}	1.25 (±0.013)^{b}	1.25 (±0.02)^{b}
	CV	3.02	2.11	1.91	3.16	1.43	2.3
	SED	1.45	1.06	0.95	0.10	1.212	2.03

Table 3. Effect of P. castaneae on growth parameters of Tomato under saline conditions

Treatments	Plant Height (cm)		No. of leaves / Plant		No. of branches per plant		First flower initiation	50 % flowering
	30DAP	60DAP	30DAP	60DAP	30DAP	60DAP		
T_1	42.33	50.33	24.67	39.67	4.33	6.00	37.67	42.33
T2	49.67	60.33	40.67	51.67	7.00	10.00	33.33	37.67
T3	50.33	62.33	43.00	54.00	9.33	9.67	33.00	37.33
T4	51.67	62.00	44.33	60.33	9.33	12.00	32.33	35.67
T5	56.67	65.67	48.67	63.00	10.67	13.33	30.67	34.67
T6	58.33	72.33	57.67	68.00	10.67	14.33	30.33	34.67
CD (5%)	2.37	1.88	2.46	3.81	2.27	4.19	2.027	1.105

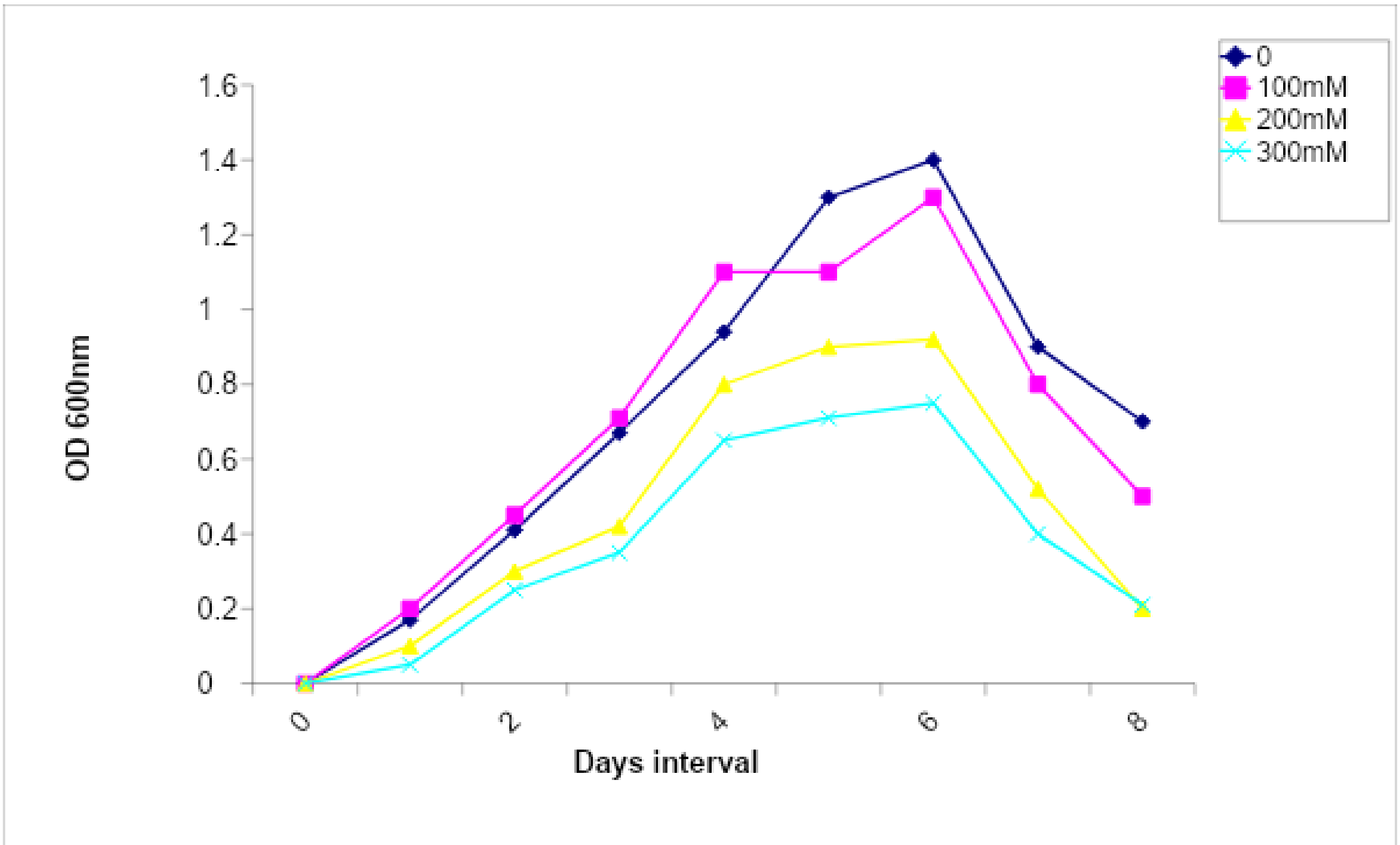
T_1– Control, T_2 – 75% recommended N + 75% recommended P, T_3 – 100% N, P and K, T_4 - 75% recommended N + 75% recommended P + *Azospirillum* + *Phosphobacteria* (Seed treatment + Soil application), T_5 - 75% recommended N + 75% recommended P + *Paenibacillus* strain (Seed treatment + Soil application), T_6 – 75% recommended N + 75% recommended P + *Azospirillum* + *Phosphobacteria* + *Paenibacillus* strain.

Table 4. Effect of P. castaneaeinoculation on yield parameters of Tomato (F1 hybrid :Sivam) under saline conditions

Treatments	Average fruit weight (g)	Average yield per plant (kg)	Plot yield (kg/15m^2)	Yield (t/ ha)
T_1	49.57	1.88	43.18	28.78
T_2	56.79	2.09	48.06	32.04
T_3	62.27	2.16	48.84	32.56
T_4	70.76	2.42	53.08	35.39
T_5	81.50	2.59	55.68	37.12
T_6	50.28	2.81	60.85	40.57
CD (5%)	3.26	0.47	3.92	3.29

T_1– Control, T_2 – 75% recommended N + 75% recommended P, T_3 – 100% N, P and K, T_4 - 75% recommended N + 75% recommended P + *Azospirillum* + *Phosphobacteria* (Seed treatment + Soil application), T_5 - 75% recommended N + 75% recommended P + *Paenibacillus* strain (Seed treatment + Soil application), T_6 – 75% recommended N + 75% recommended P + *Azospirillum* + *Phosphobacteria* + *Paenibacillus* strain.

Fig 1. Growth of Paenibacilluscastaneae (VPB1) under salinity stress.



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