



MODULATION OF MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS IN SALT-STRESSED ACID LIME (*CITRUS AURANTIFOLIA* SWINGLE) BY BENEFICIAL MICROBIAL INOCULANTS

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(Date of Receiving-23-05-2025; Date of Acceptance-30-07-2025)

ABSTRACT

Soil salinity imposes a severe threat to the plants and beneficial microbes can be a great choice to manage salt stress in acid lime (*Citrus aurantifolia* Swingle). Inoculation of *Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma viride* were used to evaluate their effect on different attributes of acid lime under artificially induced salt stress. Stress reduced shoot, root, seedling length. On the other hand, the root/shoot decreased due to salt stress as compared to non-inoculated seeds. *Pseudomonas fluorescens* followed by *Bacillus subtilis* and *Trichoderma viride* improved these different attributes and maintained a better growth and development of the acid lime plants. Salt stress also influenced the shoot, root and leaf fresh and dry weight too. The weight got decreased, while microbes still maintained a better shoot, root and leaf fresh and dry weight. Our findings suggest that these different microbial inoculants have the potential to manage salt stress in acid lime (*Citrus aurantifolia* Swingle).

Key words: Acid lime, growth, microbes, salinity.

Introduction

In spite of the technological innovations in agriculture, which dramatically increased food production in the past few decades (Godfray *et al.*, 2010), food security globally is being challenged by several factors including climate change. Large tracts of land have become unproductive or less productive due to soil salinization alone. A worldwide and ever-changing issue, soil salinization is expected to worsen in the future due to climate change scenarios, such as rising sea levels and their effects on coastal regions, rising temperatures and increased evaporation, etc. There are no precise statistics on the most recent estimates of the global extent of soils damaged by salt, and the information provided by various data sources varies (Shahid *et al.*, 2018). India's salt-affected soils are on the rise, endangering both the country's economic growth and food security. Being citrus is one of the most important fruit crops in the world and India as well, are going to be affected adverse heavily

because citrus is one of the most sensitive crops to soil salinity. So, there is a need to consider the problem of soil salinity in terms of citrus. Cations (sodium (Na^+), calcium (Ca^{+2}), and magnesium (Mg^{+2}) and anions (chloride (Cl^-), sulfate (SO_4^{-2}), and bicarbonate (HCO_3^-) are among the inorganic dissolved salts found in saline soils. These ions create an electrical field in the solution, which causes the salt concentration and the water's electrical conductivity (EC) to correlate linearly (Grattan *et al.*, 2015). Fruit yields is dropped by almost 13% for every 1.0 dS m^{-1} increase in ECs above the previous records of the threshold for EC (saturated-soil, ECs) in citrus, which was approximately 1.4 dS m^{-1} (Maas *et al.*, 1993). It is common for hazardous concentrations of Na^+ and Cl^- ions to build up inside plant cells. Cl^- ions contribute to the harmful effects of leaf abscission, growth halt, and necrosis in citrus (Li *et al.*, 2020). Fruit yield is dropped by almost 13% for every 1.0 dS m^{-1} increase in ECs above the previous records of the threshold for EC

(saturated-soil, ECs) in citrus, which was approximately 1.4 dS m^{-1} (Maas *et al.*, 1993). In order to minimize leaf ion toxicity and preserve leaf water, turgor, and photosynthesis, plants react to salt stress by raising the root/canopy ratio, chlorophyll content, and causing changes in the leaf anatomy (Acosta-Motos *et al.*, 2015). One of the sustainable methods for enhancing acid lime seedling health under salt stress is the use of beneficial microbes. PGPR participates in the following activities:

- (i) soil nutrient mobilization;
- (ii) plant growth regulator production;
- (iii) phytopathogenic attack control;
- (iv) systemic resistance induction;
- (v) soil structure improvement; and
- (vi) remediation of polluted soil.

Endophytic fungi have been shown to enhance plant growth under stress, in addition to the well-known mycorrhizal fungi and plant-growth-promoting rhizobacteria. Through improved root growth, nutrient uptake, and protection against oxidative damage, *Trichoderma* strains (endophytic fungi) increase a plant's resistance to biotic and abiotic conditions such drought and salinity (Mastouri *et al.*, 2010; Shores *et al.*, 2010). So, the aim of this study is to understand the effect of beneficial microbes on the different attributes of acid lime seedling under various levels of salt stress.

Material and Methods

At the College of Agriculture, Jabalpur, JNKVV, Jabalpur (M.P.), the experiment was conducted in 2023. The experiment was designed using a two-factorial, totally randomized design. The effectiveness of *Trichoderma viride*, *Pseudomonas fluorescens*, and *Bacillus subtilis* in managing salt stress in acid lime was evaluated. The microbial cultures were taken from Microbe Research & Production Centre situated at JNKVV, Jabalpur (M.P.). The design of the experiment was 2 factorial completely randomized design (CRD). The polyethylene bags of 6×8

inch was filled with a general composition soil, vermicompost and sand. The growing mixture weighed one kg in each bag. The sterilized seeds were dipped for nine hours in the different microbial cultures then sowed in the polyethylene bags in the net house. 2 months old seedlings with equal health and height were chosen for further experiment from every microbial inoculated pot. These pots were then treated with different salt solutions. The concentration of salt solution was gradually increased to avoid sudden osmotic shock. Then ultimately the plants were watered with 0, 3 and 6 dS m^{-1} EC. The doses were applied for 8 weeks. The plants were watered twice in a week with 150ml solution. Then the plants were evaluated for different traits.

The total 12 different treatments were applied, which are as follows: T_1 - 0 $\text{dS m}^{-1}\text{EC} \times$ No inoculation, T_2 - 3 $\text{dS m}^{-1}\text{EC} \times$ No inoculation, T_3 - 6 $\text{dS m}^{-1}\text{EC} \times$ No inoculation, T_4 - 0 $\text{dS m}^{-1}\text{EC} \times B. subtilis$, T_5 - 3 $\text{dS m}^{-1}\text{EC} \times B. subtilis$, T_6 - 6 $\text{dS m}^{-1}\text{EC} \times B. subtilis$, T_7 - 0 $\text{dS m}^{-1}\text{EC} \times P. fluorescens$, T_8 - 3 $\text{dS m}^{-1}\text{EC} \times P. fluorescens$, T_9 - 6 $\text{dS m}^{-1}\text{EC} \times P. fluorescens$, T_{10} - 0 $\text{dS m}^{-1}\text{EC} \times T. viride$, T_{11} - 3 $\text{dS m}^{-1}\text{EC} \times T. viride$, T_{12} - 6 $\text{dS m}^{-1}\text{EC} \times T. viride$

Shoot length

The shoot length was observed at the end of the experiment. After carefully removing the seedlings from the polyethylene bags, extra water was blotted out with tissue paper. The seedlings were then put on the sterile laboratory bench. For the purpose of measuring shoot length, five seedlings were selected from each replication of each treatment. Thus, it is possible to measure shoot length at the same time. The 30-cm ruler was used to measure the shoot's length. It was measured from the shoot's base, or cotyledon node, to its tip. Centimetres (cm) were used to measure the shoot length.

Root length

The same plants were utilized to measure the root length after the shoot length was determined. On the lab bench, the roots were spread out completely—not coiled

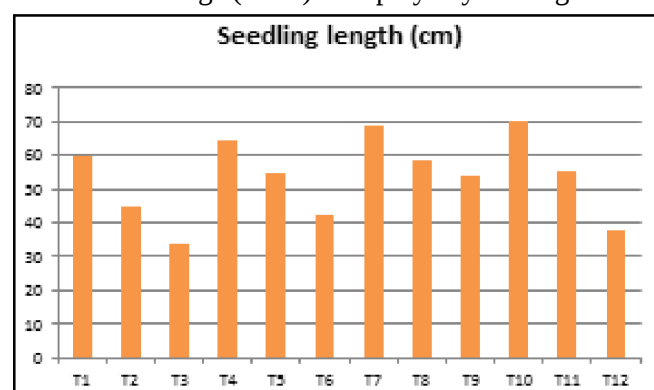


Fig. 1: Effect of different treatments on seedling length.

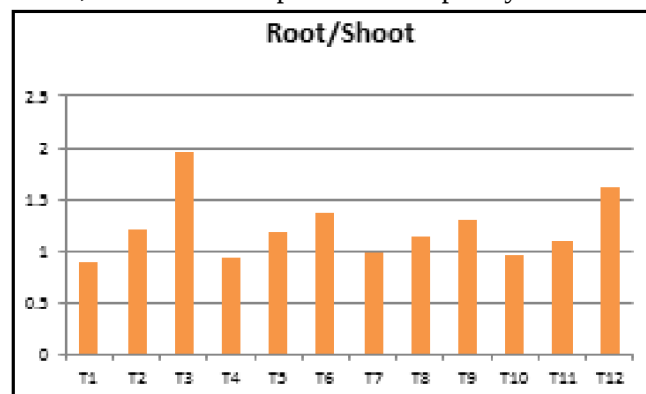


Fig. 2: Effect of different treatments on Root/Shoot.

or knotted. From the collar, where the shoot and root meet and the tip of the longest root were used to measure the root’s length. Centimeters (cm) were used to measure the root length.

Root/Shoot

Root/shoot was calculated by dividing the root length by shoot length.

Seedling length

Seedling length was calculated by measuring the overall length including shoot and root length. The unit of seedling length is also cm.

Shoot, Leaf & Root Fresh weight

By separating root and shoot of the plant, the shoot and root was measured individually through using weighing balance in the lab. The leaves were plucked from the shoot and weighed. The fresh weight was noted in g.

Shoot, Leaf & Root Dry weight

After taking the shoot, root & leaves fresh weight, the samples were kept in different envelops. These envelops were kept in the hot air oven at 60–70°C until the plants weight became constant. Then the samples were weighed on a weighing balance.

Staistical analysis

The collected data are presented as the means of the three replicates. One way analysis of variance (ANOVA) was used to determine significantly significant differences between the means of treatments according to Least significance difference test. The statistical analysis was done by using excel.

Results

Shoot length

Shoot length decreased significantly when the stress increased. *T. viride* had longest shoot (35.58 cm) under no stress, followed by *P. fluorescens* (34.75 cm) and *B. subtilis* (33.28 cm). The plants which were not treated

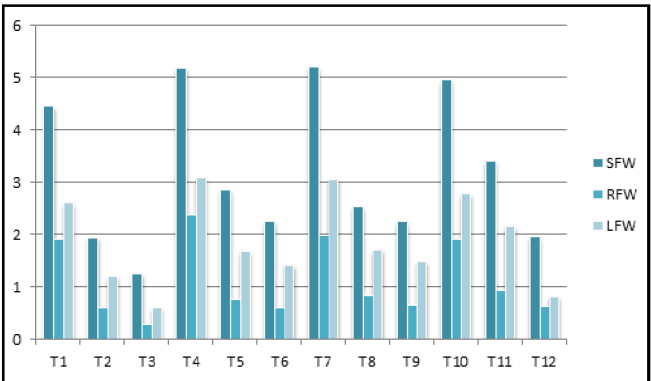


Fig. 3: Effect of different treatments on shoot, root and leaf fresh weight in acid lime.

Table 1: Effect of different treatments on shoot, root, seedling length and Root/Shoot in acid lime.

Treatments	A	B	C	D
T ₁	31.40	28.21	59.61	0.90
T ₂	20.43	24.53	44.95	1.20
T ₃	11.40	22.28	33.68	1.96
T ₄	33.28	31.32	64.60	0.94
T ₅	25.25	29.53	54.78	1.18
T ₆	17.80	24.4	42.20	1.37
T ₇	34.75	34.14	68.89	0.99
T ₈	27.30	31.36	58.66	1.15
T ₉	23.50	30.32	53.82	1.29
T ₁₀	35.58	34.32	69.91	0.96
T ₁₁	26.40	28.76	55.16	1.10
T ₁₂	14.50	23.2	37.70	1.61
SE(m)±	0.68	0.99	1.31	0.05
C.D. (P=0.05)	1.93	2.83	3.72	0.13
C.D. (P=0.01)	2.57	3.77	4.97	0.18
A: Shoot length (cm); B: Root length (cm); C: Seedling length (cm); D: Root/Shoot				

with microbes had shortest shoot length (31.40 cm) even under no stress situation. Then shoot length further reduced to 20.43 cm at moderate stress and ultimately to 11.40 cm at highest stress and were the shortest shoot length at every level of salt stress. Where, microbes outperformed even at highest EC. The results show that the microbes especially, *P. fluorescens* was able to maintain the shoot length at highest EC in acid lime, resulting in a shoot length almost double that of the uninoculated control (T₃). The stem growth was also found to be enhanced by the use of *P. fluorescens* under salinity in pomegranate (Ahmadi *et al.*, 2024). *B. subtilis* (T₆) also showed a good improvement, while *T. viride* (T₁₂) provided some benefit but less than the other two inoculants at this stress level.

Root length

Similar to shoot length, root length generally decreased with increasing salinity, though the reduction was less drastic in some inoculated treatments. All

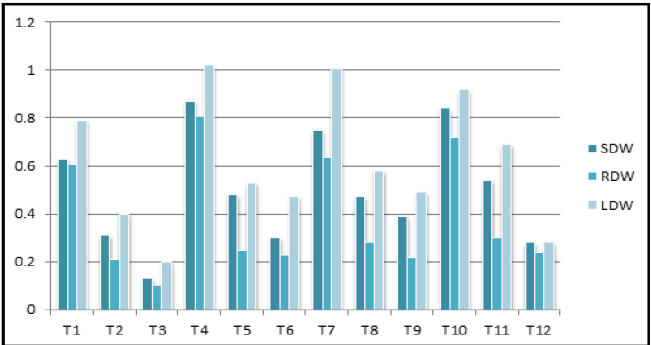


Fig. 4: Effect of different treatments on shoot, root and leaf dry weight in acid lime.

Table 2: Effect of different treatments on shoot, root and leaf fresh weight in acid lime.

Treatments	Shoot fresh weight (g)	Root fresh weight (g)	Leaf fresh weight (g)
T ₁	4.45	1.90	2.60
T ₂	1.94	0.61	1.22
T ₃	1.26	0.29	0.60
T ₄	5.19	2.39	3.09
T ₅	2.87	0.76	1.69
T ₆	2.26	0.60	1.42
T ₇	5.20	1.98	3.07
T ₈	2.54	0.83	1.72
T ₉	2.25	0.65	1.49
T ₁₀	4.95	1.92	2.78
T ₁₁	3.42	0.924	2.16
T ₁₂	1.95	0.626	0.82
SE(m)±	0.10	0.03	0.05
C.D. (P=0.05)	0.28	0.08	0.16
C.D. (P=0.01)	0.38	0.10	0.21

inoculations significantly promoted root growth under non-saline conditions, with *P. fluorescens* (T₇) (34.32 cm) and *T. viride* (T₁₀) (34.14 cm) showing the most significant enhancement. Under moderate salinity, all inoculants considerably improved root length compared to the control (T₂). *P. fluorescens* (T₈) (31.36 cm) and *B. subtilis* (T₅) (29.53 cm) were particularly effective in maintaining root growth. At high salinity, *P. fluorescens* (T₉) (30.32 cm) demonstrated a remarkable ability to sustain root length, showing the highest root length under severe stress conditions. *B. subtilis* (T₆) (24.4 cm) and *T. viride* (T₁₂) (23.2 cm) also provided some protection, but to a lesser extent than *P. fluorescens*.

Seedling length

Seedling length, being the sum of shoot and root length, followed a similar pattern of decrease with increasing salinity. *P. fluorescens* (T₇) and *T. viride* (T₁₀) significantly enhanced total seedling growth in non-saline conditions. All inoculants significantly improved seedling length under moderate salinity, with *P. fluorescens* (T₈) being the most effective in promoting overall growth. Under high salinity, *P. fluorescens* (T₉) provided the most substantial improvement in seedling length, demonstrating its strong potential in mitigating severe salt stress. *B. subtilis* (T₆) also offered considerable protection.

Root/shoot

Root/shoot continuously increased with the increase in salt concentration increases. It shows that the shoot length was more affected by the salt than the root length, which ultimately leads to higher root/shoot. Under moderate stress, non-inoculated plants had the most root/

Table 3: Effect of different treatments on shoot, root and leaf dry weight in acid lime.

Treatments	Shoot dry weight (g)	Root dry weight (g)	Leaf dry weight (g)
T ₁	0.63	0.61	0.79
T ₂	0.31	0.21	0.40
T ₃	0.13	0.10	0.20
T ₄	0.87	0.81	1.02
T ₅	0.48	0.25	0.53
T ₆	0.30	0.23	0.47
T ₇	0.75	0.64	1.01
T ₈	0.47	0.28	0.58
T ₉	0.39	0.22	0.49
T ₁₀	0.84	0.72	0.92
T ₁₁	0.54	0.30	0.69
T ₁₂	0.28	0.24	0.28
SE(m)±	0.02	0.01	0.02
C.D. (P=0.05)	0.04	0.04	0.05
C.D. (P=0.01)	0.06	0.05	0.07

shoot (1.20) while, *T. viride* had the least (1.10), showing least change as compared to *T. viride* (0.96) inoculated plants in non-saline conditions. The uninoculated plants had the highest root/shoot (1.96) at 6 ds m⁻¹ while *P. fluorescens* showed the least root/shoot showing more balanced growth under severe stress. The results show that microbes helped plants to maintain better resource allocation.

Shoot fresh weight

Shoot fresh weight significantly declined with increasing salinity, but microbial inoculation mitigated this effect. The highest shoot fresh weight was observed in T₇ (*P. fluorescens*, 0 dS m⁻¹) at 5.20 g, followed very closely by T₄ (*B. subtilis*, 5.19 g) and T₁₀ (*T. viride*, 4.95 g). These values were all higher than the uninoculated control T₁ (4.45 g), indicating a growth-promoting effect of microbial inoculation even under non-saline conditions. At 3 dS m⁻¹, T₅ (*B. subtilis*: 2.87 g), T₈ (*P. fluorescens*: 2.54 g), and T₁₁ (*T. viride*: 3.42 g) outperformed the uninoculated saline treatment T₂ (1.94 g). Similarly, at 6 dS m⁻¹, T₆ (*B. subtilis*: 2.26 g), T₉ (*P. fluorescens*: 2.25 g), and T₁₂ (*T. viride*: 1.95 g) showed much better performance than the uninoculated T₃ (1.26 g), with values nearly doubled.

Root fresh weight

Root fresh weight followed a similar pattern. The highest root fresh weight (2.39 g) was recorded in T₄ (*B. subtilis*, 0 dS m⁻¹), followed by T₇ (*P. fluorescens*: 1.98 g), and T₁₀ (*T. viride*: 1.92 g). These values were significantly higher than T₁ (1.90 g), suggesting that beneficial microbes enhance belowground biomass even

under optimal conditions. At 3 dS m⁻¹, root fresh weight improved in inoculated treatments (T₅: 0.76 g, T₈: 0.83 g, T₁₁: 0.924 g) compared to T₂ (0.61 g). At 6 dS m⁻¹, while a general decline was observed, T₆ (0.60 g), T₉ (0.65 g), and T₁₂ (0.626 g) performed better than the uninoculated control T₃ (0.29 g).

Leaf fresh weight

Leaf fresh weight was also influenced by both salinity and microbial treatment. The maximum leaf fresh weight was recorded in T₄ (*B. subtilis*, 3.09 g) and T₇ (*P. fluorescens*, 3.07 g), followed by T₁₀ (*T. viride*, 2.78 g), all higher than the uninoculated control T₁ (2.60 g). Under 3 dS m⁻¹, inoculated treatments (T₅: 1.69 g, T₈: 1.72 g, T₁₁: 2.16 g) were significantly better than the uninoculated T₂ (1.22 g). At 6 dS m⁻¹, microbial inoculation again helped retain higher leaf fresh weight—T₆ (1.42 g), T₉ (1.49 g), and T₁₂ (0.82 g) all performed better than T₃ (0.60 g).

Shoot dry weight

Shoot dry weight was significantly influenced by salinity levels and microbial inoculation. The highest shoot dry weight (0.87 g) was recorded in T₄ (0 dS m⁻¹ × *B. subtilis*), followed closely by T₁₀ (0.84 g; 0 dS m⁻¹ × *T. viride*), and T₇ (0.75 g; 0 dS m⁻¹ × *P. fluorescens*), all of which were significantly superior to the uninoculated control (T₁: 0.63 g). As salinity increased to 3 and 6 dS m⁻¹, shoot dry weight declined across all treatments. At 6 dS m⁻¹, microbial inoculation still provided mitigation: T₆ (*B. subtilis*) and T₉ (*P. fluorescens*) showed higher shoot dry weights (0.30 g and 0.39 g, respectively) compared to the non-inoculated control T₃ (0.13 g). *Trichoderma viride* at 6 dS m⁻¹ (T₁₂) was less effective, yielding only 0.28 g.

Root dry weight

Root dry weight followed a similar trend. The maximum root dry weight (0.81 g) was again observed in T₄ (*B. subtilis*, 0 dS m⁻¹), followed by T₁₀ (*T. viride*, 0.72 g), and T₇ (*P. fluorescens*, 0.64 g). Under salt stress, microbial inoculants improved root dry weight compared to non-inoculated treatments. At 3 dS m⁻¹, inoculated treatments like T₅ (*B. subtilis*: 0.25 g), T₈ (*P. fluorescens*: 0.28 g), and T₁₁ (*T. viride*: 0.30 g) outperformed the non-inoculated T₂ (0.21 g). At 6 dS m⁻¹, *B. subtilis* (T₆) and *T. viride* (T₁₂) had similar root dry weights (0.23–0.24 g), while *P. fluorescens* (T₉) showed slightly lower performance (0.22 g) but was still better than the uninoculated T₃ (0.10 g).

Leaf dry weight

The pattern of leaf dry weight mirrored that of shoots and roots. The highest leaf dry weight was observed in

T₄ (*B. subtilis*, 1.02 g), followed closely by T₇ (*P. fluorescens*, 1.01 g) and T₁₀ (*T. viride*, 0.92 g). In contrast, T₃ (6 dS m⁻¹ without inoculation) recorded the lowest value (0.20 g). Microbial inoculation helped retain leaf biomass under salt stress. At 3 dS m⁻¹, T₅ (0.53 g), T₈ (0.58 g), and T₁₁ (0.69 g) all performed better than T₂ (0.40 g). At 6 dS m⁻¹, *P. fluorescens* (T₉: 0.49 g) and *B. subtilis* (T₆: 0.47 g) were more effective than *T. viride* (T₁₂: 0.28 g), though all were superior to the uninoculated control (T₃: 0.20 g).

Discussion

According to reports, plants under salt stress have nutritional and osmotic imbalances, which lower photosynthesis and ultimately impede plant development (Pandit *et al.*, 2024). Studies have already shown that salinity, not only reduce shoot length, it reduces root biomass too in citrus (Al-Yassin *et al.*, 2004). Significant morphological changes in the responses of plant development can result from excessive salt in the soil also. The escalation in osmotic stress and decreased cell water content can be the reason for the reduction in the shoot length (Souri *et al.*, 2021). Xi *et al.*, 2022 found the same effect of salt stress on root dry weight, shoot dry weight and root/shoot in *Juglans microcarpa* L. Different salinity dosages were found to have a substantial effect on tomato plant height (Madugundu *et al.*, 2023). Higher concentrations of sodium chloride (NaCl) significantly reduced the number of sprouts, plant height, root weight, plant weight, and leaf weight of aloe vera plants too (Moghbeli *et al.*, 2012). Improved root development and more efficient nutrient uptake can be the reasons behind microbes helping seedlings grow well under different levels of salinity. *P. fluorescens*-treated plants showed a marked increase in stem length and shoot fresh weight of soybean under salinity (Abulfaraj *et al.*, 2021). Lastochkina *et al.*, 2017 also reported that in comparison to non-saline settings, wheat plant growth (seedling length, and fresh and dry weights) was reduced following salt stress treatment. The growth parameters of wheat seedlings subjected to salt stress are protected when seeds were treated with *B. subtilis* prior to sowing. In comparison to the control plant, salt concentrations inhibited the tomato plants' shoot height, root length, and the fresh and dry weight of their shoots and roots but compared to plants treated with *T. viride*, plants not treated with the fungi exhibited a larger decrease in these parameters (Metwally *et al.*, 2023).

Conclusion

In conclusion, this research unequivocally demonstrates the significant potential of microbial

interventions in mitigating salt stress in acid lime (*Citrus aurantifolia* Swingle). Our findings highlight that specific microbes, when applied to acid lime plants under saline conditions, effectively enhance various growth parameters. The study revealed that inoculated plants exhibited improved morphological traits. While this study provides compelling evidence for the efficacy of microbial applications, further research is warranted to elucidate the precise molecular interactions between the selected microbes and acid lime under saline conditions. Future investigations could also focus on optimizing application methods, exploring the long-term effects of microbial inoculation in field conditions, and assessing the economic viability of such interventions for large-scale acid lime production in salt-affected regions. Nevertheless, the present work lays a strong foundation for developing sustainable and eco-friendly strategies for salt stress management in acid lime, contributing significantly to enhancing agricultural resilience in challenging environments.

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