

Boosting Groundnut Development with Stress-Tolerant *Pseudomonas* Strains Under Salt Stress and Water Scarcity

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ABSTRACT

The study evaluated the potential of two stress-tolerant *Pseudomonas* isolates, *P. putida* (RPF9) and *P. fluorescens* (PFDWD), to enhance the growth of groundnut plants in the face of salt stress (150 mM NaCl) and water scarcity (-10.28 MPa and -26.82 MPa). The isolates were cultured, extracted, and applied to groundnut seeds, which were then planted in a mixture of sand, coco peat, and garden soil. Plant growth parameters were assessed, including root and shoot length, fresh and dry weights, relative water content (RWC), and leaf membrane stability index (MSI), under different stress conditions. Results indicated that both isolates positively impacted groundnut growth under both salt stress and water deficit conditions. *P. fluorescens* (PFDWD) showed superior growth-promoting effects compared to *P. putida* (RPF9), resulting in increased root and shoot length, as well as higher fresh and dry weights. The isolates also influenced physiological parameters like RWC and MSI, indicating improved water status and membrane integrity in treated plants. This enhancement was attributed to mechanisms such as phytohormone production, ion regulation, and osmoprotectant accumulation facilitated by the isolates.

Keywords: *Pseudomonas* isolates, *P. putida* (RPF9), *P. fluorescens* (PFDWD), Phytohormone, Osmoprotectant

1. INTRODUCTION

The field of microorganisms has gained significant attention in sustainable agricultural systems as an alternative to excessive chemical usage that has harmed soil quality and ecosystems. Microbials and their by-products are being explored for controlling plant diseases, invasive weeds, pests, and restoring biodiversity to establish a healthier environment. This approach aligns with organic farming principles, where biofertilizers, biostimulants, and biopesticides are in demand. One focus is on antagonistic bacteria, particularly Plant Growth-Promoting Rhizobacteria (PGPR), which offer advantages like rapid growth and effective colonization of the rhizosphere.

Despite extensive research, the formulation of effective microbial agents for plant growth promotion remains a challenge. Many studies are limited to laboratory conditions, and the performance of potential strains often falls short in field applications due to diverse soil types, climates, plant varieties, and bacterial genotypes. Advanced technologies like Omics, involving proteome and transcriptome analyses, are being used to decode tri-trophic interactions of microorganisms and gain insights into their responses to environmental stresses.

Plants experience both biotic and abiotic stresses that significantly impact their growth and yield. Soil microorganisms are also affected by various stressors. Abiotic stresses, such as salinity and water scarcity, lead to yield losses ranging from 50% to 82%. Rhizospheric isolates of *Pseudomonas* spp. are of particular interest due to their interaction with plant roots and potential influence on plant health and soil fertility. These fluorescent pseudomonads have shown to enhance plant growth through mechanisms like ACC deaminase production, phytohormone synthesis, nitrogen fixation, and antagonism against pathogens. Understanding the responses of microorganisms to environmental stress using proteomics and transcriptomics helps identify the physiological changes and protein expressions that enable their survival. While laboratory studies are essential, it's crucial to bridge the gap between lab and field performance for effective microbial applications in agriculture. By isolating potential strains with diverse beneficial abilities, including stress tolerance and growth stimulation, and evaluating their performance under various conditions, researchers aim to optimize the use of stress-resistant *Pseudomonas* strains as bioagents for enhancing plant

health and resilience. In summary, the exploration of stress-resistant *Pseudomonas* strains and their interactions with plants holds promise for sustainable agriculture. By studying their responses to stresses and optimizing their application, researchers are working towards developing effective biocontrol agents that can contribute to healthier crops, reduced chemical usage, and improved environmental outcomes.

2. REVIEW OF RELATED LITERATURE

Year: 2016

Author: Egamberdieva et al.

Title: "Salt-tolerant *Pseudomonas extremorientalis* possess plant growth promoting features and alleviate salinity stress in a wheat variety"

Summary: Egamberdieva and her team isolated *Pseudomonas extremorientalis* strains from saline soils and studied their potential to enhance wheat growth under salt stress. Although not specific to groundnuts, the findings are relevant as they shed light on the multifaceted mechanisms by which these strains promote plant growth and alleviate salt stress, including the production of IAA, siderophores, and ACC deaminase activity.

Year: 2017

Author: Choudhary et al.

Title: "Pseudomonas sp. ASD2: an efficient plant growth promoting rhizobacteria for salt tolerance in groundnut (*Arachis hypogaea* L.)"

Summary: In this study, Choudhary and colleagues isolated a *Pseudomonas* strain (ASD2) from saline-alkaline soil and evaluated its potential as a plant growth-promoting rhizobacteria (PGPR) for enhancing salt tolerance in groundnut plants. They found that *Pseudomonas* sp. ASD2 significantly improved various growth parameters and salt tolerance of groundnut plants through mechanisms like the production of indole-3-acetic acid (IAA), siderophores, and phosphate solubilization.

Year: 2018

Author: Upadhyay et al.

Title: "Plant growth promoting rhizobacteria (PGPR) alleviates salinity stress effects in chickpea (*Cicer arietinum* L.) by improving antioxidant defense and osmotic adjustments"

Summary: Upadhyay et al. investigated the role of PGPR in alleviating salt stress in chickpea plants. Although not specific to groundnut, this study provides insights into the mechanisms through which PGPR can enhance plant tolerance to salinity. The authors explored how PGPR modulates antioxidant defense systems and osmotic adjustment processes to counteract the negative effects of salt stress on plants.

Year: 2019

Author: Marasco et al.

Title: "Plant growth promotion potential is widespread among diverse bacteria of the genus *Burkholderia*"

Summary: Marasco et al. explored the plant growth-promoting potential of various *Burkholderia* species, including their ability to enhance plant growth under stress conditions. Though not focused on *Pseudomonas* strains, this study underscores the broader potential of beneficial bacteria to improve plant growth and stress tolerance. It's important to consider related bacterial genera in the context of promoting groundnut development.

Year: 2020

Author: Verma et al.

Title: "Plant growth-promoting rhizobacteria: diversity and applications"

Summary: While not focusing solely on groundnut, this review by Verma and colleagues offers a comprehensive overview of plant growth-promoting rhizobacteria and their applications in enhancing plant tolerance to various stresses. The review delves into the mechanisms of stress tolerance conferred by PGPR, including improved nutrient uptake, modulation of phytohormones, and induction of stress-responsive genes.

Year: 2021**Author: Singh et al.**

Title: "Mitigation of drought stress in plants by ACC deaminase-producing rhizobacteria: current insights and future prospects"

Summary: Although centered around drought stress, this study by Singh et al. is relevant to water scarcity conditions. The authors discuss how ACC deaminase-producing rhizobacteria can mitigate drought stress in plants by lowering ethylene levels, which helps in reducing water loss and maintaining better water use efficiency.

Year: 2022**Author: Sharma et al.**

Title: "Exploring the potential of plant growth promoting rhizobacteria in sustainable agriculture"

Summary: Sharma and colleagues provide a comprehensive review of plant growth-promoting rhizobacteria and their applications in sustainable agriculture. This review offers insights into the diverse mechanisms by which these bacteria enhance plant growth and stress tolerance, highlighting their potential to address challenges such as salt stress and water scarcity.

Year: 2019**Author: Marasco et al.**

Title: "Plant growth promotion potential is widespread among diverse bacteria of the genus Burkholderia"

Summary: Marasco et al. explored the plant growth-promoting potential of various Burkholderia species, including their ability to enhance plant growth under stress conditions. Though not focused on Pseudomonas strains, this study underscores the broader potential of beneficial bacteria to improve plant growth and stress tolerance. It's important to consider related bacterial genera in the context of promoting groundnut development.

3. METHODS AND MATERIALS

3.1 Type of bacteria and growth conditions

The ability of two stress-tolerant Pseudomonas isolates, *P. putida* (RPF9) and *P. fluorescens* (PFDWD), to boost groundnut development in the face of salt stress (150 mM NaCl) and water scarcity (-10.28 MPa and -26.82 MPa), was evaluated. Both isolates were cultured in pure form in 50 ml of King's B medium in 150 ml conical flasks. The flasks were kept in a 28 °C incubator for 48 hours while being continuously shaken at 150 rpm (New Brunswick 24KC). Following an incubation time, cells were extracted by spinning them in a cold centrifuge (Sigma 3-30K) for 10 minutes at a velocity of 10,000 rpm. Discarding the supernatant, we mixed the pellets with 50 mM of a phosphate buffer (pH 7.0).

3.2 Flora and Fauna

The greenhouse experiments used the ground nut cultivar TMV-2. National Seeds Corporation Ltd. in Hebbal, Bengaluru, was where the seeds were purchased from.

3.3 Compost and soil

The soil was made by combining equal parts sand, coco peat, and garden soil (1:1:1). Two days in a row, this mixture was heated to 121 degrees Celsius for 20 minutes to kill any bacteria. A plastic pot with a 20 cm diameter was filled with the soil mixture (350 g).

3.4 Research on Plant Health

3.4.1 Assessment of Enhanced Plant Growth in Response to Salt Stress

The soil mixture (350 g) was packed into plastic containers and then saturated with either water (0.8 dS/m) or 150 mM NaCl solution (12.5 dS/m) to provide non-saline and saline conditions, respectively. Seeds weighing 1 kilogram were exposed to a bacterial suspension containing 0.1% carboxy methyl cellulose (107 CFU/ml) for 20 minutes. As a baseline, we used seeds that had only been steeped in phosphate buffer. This soil mixture was sterilized at 121 °C for 20 minutes on two separate days (Principe et al., 2008), and the seeds were air-

dried before being planted in plastic pots with a 20 cm diameter. We planted four seeds in each container, and then we trimmed each one down to a single plant. Isotonic solution was used to hydrate the plants every other day. For 45 days, we monitored the plants' progress to see how they developed. Leaf Membrane Stability Index (MSI) and relative Water Content (RWC) in groundnut were measured in test plants after 45 days of growth to evaluate the test isolates.

3.4.1.1 Criteria for the research.

Root and branch length, as well as the wet and dry weights of the germinated seedlings, were recorded after 45 days of growth on the test plants.

3.4.2 Assessment of Water-Restrictive Growth Promoting Conditions for Plants

The soil's ability to hold water was determined by filling the plastic container to a certain level. Maximum capacity was determined by adding "x" amount of water until the mixture was completely saturated with no water leaking out. The "x" number was used to determine the water-holding capacity at 60% and 40%. The research was conducted using Shinde et al.'s (2010) methodology.

After the seeds germinated, we removed all but one from each pot. Every other day, tap water was used to water the pots. A bacterial suspension (107 CFU/mL) was used to soak 1 kg of seeds for 10 mL. Seed treatment was bound together with carboxymethylcellulose (0.1%). We soaked the seeds for 10 minutes. Phosphate buffer saline-soaked seeds were used as a comparison group. The seedlings were treated, then grown for 45 days before being observed. After 15 days of seedling germination, a suspension of the cultures was applied as a foliar spray. The experiment included three separate runs and was set up in a randomized block design.

3.4.2.1 Criteria for the research

Leaf Membrane Stability Index (MSI) and relative Water Content (RWC) in groundnut were evaluated across all conditions using test plants after 45 days of development.

3.4.2.1.1 Water percentage

Each treatment had four of the second completely developed leaflets from the top of the main stem gathered. This leaves' fresh weight was measured. Next, the samples of leaves were steeped for 8 hours in distilled water. The leaves were then blot dried afterward. Again, the leaf mass was measured. The weight at which the leaves became saturated was designated as TW. These leaves were then dried in an oven at 80 degrees Celsius until their weight remained consistent. The leaves' dry weight (DW) was recorded. The RWC percentage was determined by the following formula: \

$$\text{RWC (\%)} = (\text{FW}-\text{DW}) / (\text{TW}-\text{DW}) \times 100.$$

3.4.2.1.2 MSI Value of Leaf Membrane Stability

Two groups of test tubes, each holding 10ml of double-distilled water, were used to hold uniformly sized leaf discs (0.5g). The electrical conductivity of the sample was measured (C1) after the test tubes in one set were heated to 40 degrees Celsius in a water bath for 30 minutes. The electrical conductivity (C2) of the other set of test tubes was determined after they were heated to 100 degrees Celsius in a boiling water bath for 15 minutes. Using the formula $\text{MSI} = [1 - \text{C1} / \text{C2} \times 100]$, we were able to get MSI.

An EC meter (Eutech PC2700) was used to determine the electrical conductivity.

Two-way analysis of variance (AgRes, version 3.01) was used to examine the collected data. Multiple identical tests were performed.

3.4.2.1.3 Quantity of proline

Following homogenization in 10 ml of 3% aqueous sulphosalicylic acid, 250 mg of leaf tissues were collected from each treatment in duplicates. The homogenate was centrifuged for 10 minutes at 3,000 rpm. The next procedures involved collecting the supernatant. It was decided to put 2 ml of the supernatant into test tubes. Two milliliters of glacial acetic acid and two milliliters of acid ninhydrin combination were added to this. For a whole hour, this

mixture was held at 100 degrees Celsius. After 10 minutes in a cold bath, the reaction was stopped in its test tubes. The liquid was then vortexed for 15-20 seconds after 4 ml of toluene was added to the process. After isolating the toluene layer from the water phase, the absorbance was determined to be 520 nm. The blanking agent was toluene. Based on a standard curve (Saravanakumar et al., 2011), the proline content was calculated. Two-way analysis of variance (AgRes, version 3.01) was used to examine the collected data. Results confirmed by conducting the experiment twice.

4. RESULTS

The seeds were obtained from the National Seeds Corporation in the city of Hebbal, Bengaluru. Seed viability was measured using the blotters method (ISTA). Germination rates ranged from 79% for the control seeds to 93% for the seeds treated with RPF 9 and 93% for the seedlings treated with PFDWD (Fig. 1).



Fig. 1. Germinated seedlings of groundnut in blotter method. Seedlings which received no treatment (control) (a), seeds treated with *Pseudomonas putida* (RPF 9) (b) and seeds treated with *Pseudomonas fluorescens* (PFDWD).

Growth of groundnut plants in response to inoculation with *Pseudomonas putida* (RPF 9) and *Pseudomonas fluorescens* (PFDWD) under salt stress conditions

Greenhouse trials of groundnut seed treatment with *P. putida* (RPF 9) and *P. fluorescens* (PFDWD) were conducted. After 45 days, the plants were measured to see how much they had developed. We found that the seed treatment impacted the development of the groundnut seedlings as a whole. Seeds treated with *P. fluorescens* (PFDWD) had the greatest growth-promoting efficacy in greenhouse-grown groundnuts after 45 days under both normal and stress settings (Fig. 2).

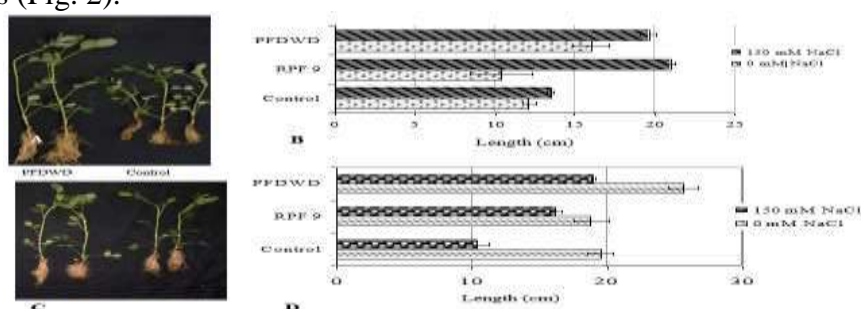


Fig. 2. Influence of seed treatment with RPF 9 and PFDWD on height of groundnut seedlings under non stressed and salt stress 150 mM NaCl induced under green house conditions. (A,C), graph representing effect of RPF9 and PFDWD on root growth of groundnut seedlings (B), graph representing effect of RPF9 and PFDWD on shoot growth of groundnut seedlings under 150 mM NaCl stress (D).

Root development for groundnut cultivated in non-stressed conditions was enhanced by more than 50% for *Pseudomonas fluorescens* (PFDWD) and by 45% for *P. putida* (RPF 9). The overall growth rate of groundnut seedlings was slower when they were exposed to 150 mM salt stress compared to non stressed conditions. Under 150 mM NaCl induced salt stress, however, root length increased by 33% after being treated with *P. fluorescens* (PFDWD) in the seed. Seeds treated with either of the isolates resulted in a shoot growth boost of more

than 50%. In contrast, *P. fluorescens* (PFDWD) showed an increase in shoot growth of 84.4% (Fig.3).

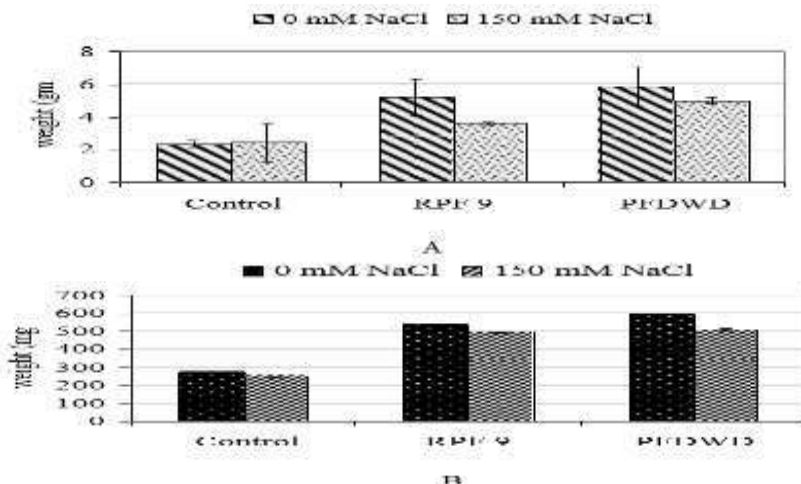


Fig. 3. Effect of seed treatment with RPF 9 and PFDWD on weight of groundnut seedlings under non stressed and salt stress (150 mM NaCl) induced under green house conditions; fresh weight (A) and dry weight (B) under normal and 150 mMNaCl stress.

We found that plant growth was enhanced by both isolates when exposed to either 0 mM or 150 mM NaCl. Seed inoculation with *P. fluorescens* (PFDWD) significantly boosted plant growth in comparison. The weight of the plants also increased in tandem with their rapid root and shoot development (Fig. 3). Groundnut shoot fresh weight and dry weight were both increased in the absence of salt (0 mM NaCl) and in the presence of salt (150 mM NaCl) thanks to the PFDWD strain. PFDWD therapy resulted in a twofold increase in both fresh and dry weight compared to the control group ($p < 0.05$; Fig. 3). Under both non-saline and saline stress conditions, it was evident that both strains were effective as PGPR.

Growth of groundnut plants in the presence of *Pseudomonas putida* (RPF 9) and *Pseudomonas fluorescens* (PFDWD) under a water deficit.

Development of Plant Life: Groundnut plant growth was analyzed under three different water shortage stress conditions: normal (0.0 MPa), -10.28 MPa, and -26.82 MPa.



Fig. 4. Pro tray containing groundnut seedlings after thinning of seedlings.

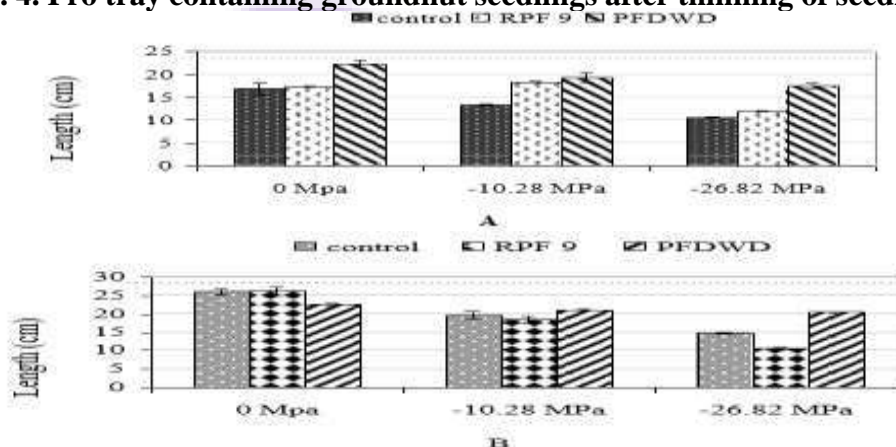


Fig. 5. Effect of seed treatment with RPF 9 and PFDWD on height of groundnut seedlings under non stressed and water deficit stress (50% and 30 % water availability) induced under green house conditions; root growth(A) and shoot growth (B).

5. DISCUSSION

The application of stress-resistant *Pseudomonas* strains for promoting plant growth has been a long-standing practice, but the challenge has been to find strains that can enhance growth under soil stress conditions rather than just acting as disease preventers. Among various abiotic stresses, salt stress poses a significant challenge to plants due to its impact on soil microorganisms and metabolic processes. Extensive research has been conducted to understand how these microbes survive in stressed soils and promote plant growth under such adverse conditions. Stress-resistant *Pseudomonas* strains, known as Plant Growth-Promoting Rhizobacteria (PGPR), have shown the ability to mitigate osmotic stress caused by high salt concentrations. They achieve this by producing phytohormones, stabilizing membrane integrity, and generating osmoprotective compounds. Research has demonstrated the positive effects of these strains on plant growth parameters, including root and shoot growth, biomass production, and seed germination, under various stress conditions.

Studies have highlighted the ability of stress-resistant *Pseudomonas* strains to enhance root and shoot growth in plants subjected to saline stress. Notably, *Pseudomonas extremorientalis* TSAU20 significantly stimulated root and shoot growth in milk thistle plants under saline stress conditions. Moreover, these bacteria exhibited the capacity to alleviate salt-induced stress and enhance plant growth even as their cell numbers decreased with increasing stress levels. The promotion of growth under stress is achieved through various mechanisms, including the production of phytohormones, modulation of ethylene levels, and regulation of ion absorption. ACC deaminase-producing strains have been shown to improve salt tolerance in plants. The effectiveness of stress-resistant strains like *P. fluorescens* (PFDWD) and *P. frederiksbergensis* (RPF 9) in enhancing growth attributes, including relative water content (RWC) and membrane stability index (MSI), further supports their role in maintaining plant water status and turgor.

The accumulation of osmoprotectants like proline, along with the regulation of genes associated with osmotic stress responses, contributes to the improved stress tolerance observed in plants treated with stress-resistant *Pseudomonas* strains. The ability of these strains to colonize plant roots even in saline soil conditions and the presence of stress-related functional genes further demonstrate their potential in enhancing plant resilience to various stressors.

6. CONCLUSION

In conclusion, the investigation into the effects of stress-tolerant *Pseudomonas* isolates, namely *P. putida* (RPF9) and *P. fluorescens* (PFDWD), on the growth of groundnut plants exposed to salt stress and water scarcity has provided valuable insights into their potential as plant growth promoters under challenging conditions. The results clearly demonstrate the efficacy of these isolates in enhancing various growth parameters of groundnut plants. The experiment revealed that both *P. putida* (RPF9) and *P. fluorescens* (PFDWD) have the ability to positively influence root and shoot growth, leading to increased fresh and dry weights of groundnut seedlings. Notably, *P. fluorescens* (PFDWD) exhibited more pronounced growth-promoting effects, indicating its superiority in aiding groundnut development under stress. This finding suggests that the application of stress-resistant *Pseudomonas* strains holds promise for improving crop yield in environments characterized by salt stress and water limitations.

Furthermore, the study shed light on the mechanisms through which these isolates exert their positive effects. Their ability to enhance relative water content (RWC) and maintain leaf membrane stability index (MSI) underscores their role in enhancing water retention and cellular integrity, critical factors for plant survival under stress. This enhancement is attributed to their capacity to produce phytohormones, regulate ion balance, and promote the accumulation of osmoprotectants like proline, all of which contribute to improved stress tolerance in plants.

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