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EVALUATION OF SELENIUM-MOBILIZING MICROBES FROM SELENIFEROUS SOILS OF PUNJAB, INDIA, FOR ENHANCING DROUGHT TOLERANCE IN RICE

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ABSTRACT

This study assessed selenium distribution in seleniferous soils of northwestern Punjab, India, and evaluated native selenium-mobilizing microbes for enhancing drought tolerance in rice. Four efficient selenium mobilizing microbial isolates were selected based on their solubilization efficiency. A pot experiment (kharif 2023–2024) was conducted to assess their performance under moisture stress. Inoculation with selenium-mobilizing microbes along with Se (20 g ha^{-1}) significantly improved plant growth, physiology, nutrient status, and yield. The best treatment showed the highest soil water content 78.87% and 84.60%, respectively under both stressed and non stressed conditions. The treatment also produced highest grain yield of 22.90 g plant-1 in non stressed and 16.53 g plant-1 in stressed condition corresponding to a minimum reduction of 27.81% due to moisture stress, comparing 41.58% recorded under control.

KEYWORDS: Selenium-mobilizing microbes, Seleniferous soils, Drought tolerance, Rice (*Oryza sativa L.*), Bacillus subtilis, Selenium bioavailability.

INTRODUCTION

Rice (*Oryza sativa L.*) is one of the major food crop globally ranked third after wheat and maize in terms of production and is the staple food crop for nearly two-third of the world's population (Abbade et al. 2021). About 52% of net sown area in India falls under rainfed agriculture, contributing 46% of food grain production and supporting livelihood for 40% of the population in the country (NRAA- National Rainfed Area Authority, New Delhi, 2020). Because the livelihood of a majority of the world's population depends on rice, it is vital to investigate how abiotic stress influences the variability of rice yields (Song et al. 2022). Rice productivity will be reduced by 200–600 kg ha-1 due to variations in drought intensity and frequency (up to 20%) by 2050 (Ramirez et al. 2018).The uneven distribution of the Indian monsoon often causes uncontrolled runoff and water scarcity issues, which negatively affect crop growth and water productivity, especially for major food crops such as rice and wheat (Birthal et al. 2015).

Bacteria play an important role in the biogeochemical cycle of selenium in nature. During bacterial metabolism, selenium is transformed by different processes (oxidation, reduction or methylation), and through selenium respiration in selenium tolerant bacteria associated with the mechanisms of assimilation and metabolization of this metalloid inside the cells. This has shown a great potential for use in bioremediation and phytoremediation of selenium contaminated sites (Tong et al. 2014). Microorganisms play a vital role in transforming inorganic Se compounds into insoluble elemental Se (Se0), which can be filtered out of drainage water (Zhou et al. 2020). Use of selenium tolerant bacteria appear as a possible alternative for selenium enhancement of cereals grown on soils with low selenium concentration. Application of selenium and microorganisms (Selenorhizobacteria) pelleted seeds when applied in soil or the rhizosphere can stimulate natural processes to enhance nutrient uptake, productivity, abiotic stress tolerance and crop quality. Selenium plays a vital role as protectant against different abiotic stresses including heavy metals, chilling, submergence, drought, and nutrient deficiency (Khan et al. 2018). Therefore, Se supplementation is needed in order to reduce the harmful effects on plant metabolism and physiology caused by drought stress can be decreased by Se supplementation. Considering this, the present work was undertaken to evaluate the efficiency of selenium-mobilizing microbes, both individually and in combination with selenium, in mitigating induced moisture stress in rice and assessing their effects on relative soil moisture content,

microbial population, rice biomass, and grain yield under drought conditions.

MATERIALS AND METHODS

A survey was conducted in seleniferous northwestern regions of Punjab, India, and 100 surface soil samples (0–15 cm) were collected from seven villages across Shaheed Bhagat Singh Nagar and Hoshiarpur district during December 2023. Samples (~250 g) were collected in sterile bags, transported under cooled conditions, and stored at 10 °C. Selenium concentration was determined using graphite furnace atomic absorption spectrometry (GFAAS). Selenium mobilization efficiency was assessed in broth supplemented with sodium selenite and selenate, and soluble and volatilized selenium were quantified using ICP-OES. For isolation of selenium-mobilizing bacteria, 10 g soil samples were enriched in tryptone soya broth and plated on selenium-amended agar (50 mg L^{-1}). After incubation at 37 °C for 24 h, red colonies indicating selenium reduction were purified and maintained. A total 14 isolates were isolated from above soil samples and on the basis of the selenium mobilizing efficiency four efficient selenium mobilizing isolates were identified and further evaluated to induce drought tolerance in rice under a pot culture experiment. The above four isolates were characterized morphologically and biochemically (Gram staining, catalase, TSI, urease, citrate, indole, methyl red, gelatin liquefaction). Further, a pot experiment was conducted with above four selenium mobilizing microbial isolates (SMMI) under open microcosm conditions with nine treatments and six replications in a factorial completely randomized design at the Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, during the Kharif season of 2024 using rice (*Oryza sativa L.*) variety Chhattisgarh 1919. To evaluate their effectiveness with and without selenium to enhance plant resistance to high moisture stress, and to assess their effect on biomass and yield of rice crop. Fresh broth cultures of four isolates viz. - SMMI-1, SMMI-2, SMMI-3, and SMMI-4 were prepared and applied to the root zone at 7 days after sowing, while selenium was applied at 20 g ha^{-1} at 15 days after sowing as per treatments. Treatments included control (T_1), individual microbial isolates (T_2–T_5), and isolates combined with selenium (T_6–T_9). The isolates originated from Punjab villages (Rakkar, Jainpur, and Mehindpur), and a fertilizer dose of N:P:K = 120:60:40 kg ha^{-1} was applied. Observations included microbial count ($\times 10^6$ g^{-1} soil), relative water content (RWC), plant dry biomass, and grain and straw yield. Statistical analysis was performed using ANOVA under factorial CRD as per Gomez and Gomez.

RESULT AND DISCUSSION

Soil sampling was carried out across the seleniferous northwestern regions of Punjab. Fourteen selenium-mobilizing microbes were isolated from the collected soil samples and evaluated for their selenium solubilization efficiency. Based on their performance, four promising isolates were selected for a pot experiment in rice to assess their role in enhancing drought tolerance under moisture stress conditions. During characterization of isolates colonies were found circular with entire margins, convex elevation, smooth moist surface, and exhibited distinct red-pink pigmentation. Gram staining revealed Gram-positive, rod-shaped cells with uniform morphology. Biochemical analysis showed their positive reactions for methyl red, citrate utilization, gelatin hydrolysis, urease, and catalase, while indole production, Voges–Proskauer test, and H_2S production were negative. These isolate were also sensitive to antibiotics.

All soil samples examined in this study for isolation of selenium mobilizers were found to be alkaline and to contain between 6.32 and 30.10 mg/kg selenium. The selenium levels in soil as estimated by using neutron activation. A total of fourteen soil samples were analyzed, corresponding to Serial Numbers 1 to 14. The measured selenium concentrations indicating substantial variation among the samples. Sample 1 recorded the minimum selenium concentration (6.32 mg/L). Among these, sample 14 recorded the highest selenium concentration (30.10 mg/L), suggesting enhanced selenium
mobilization. Hence, the isolates isolated from the sample 11-14 were taken for further study as the samples contained higher selenium content so, with this hypothesis that the isolated strains could mobilize significant amount of selenium which should have potential use.

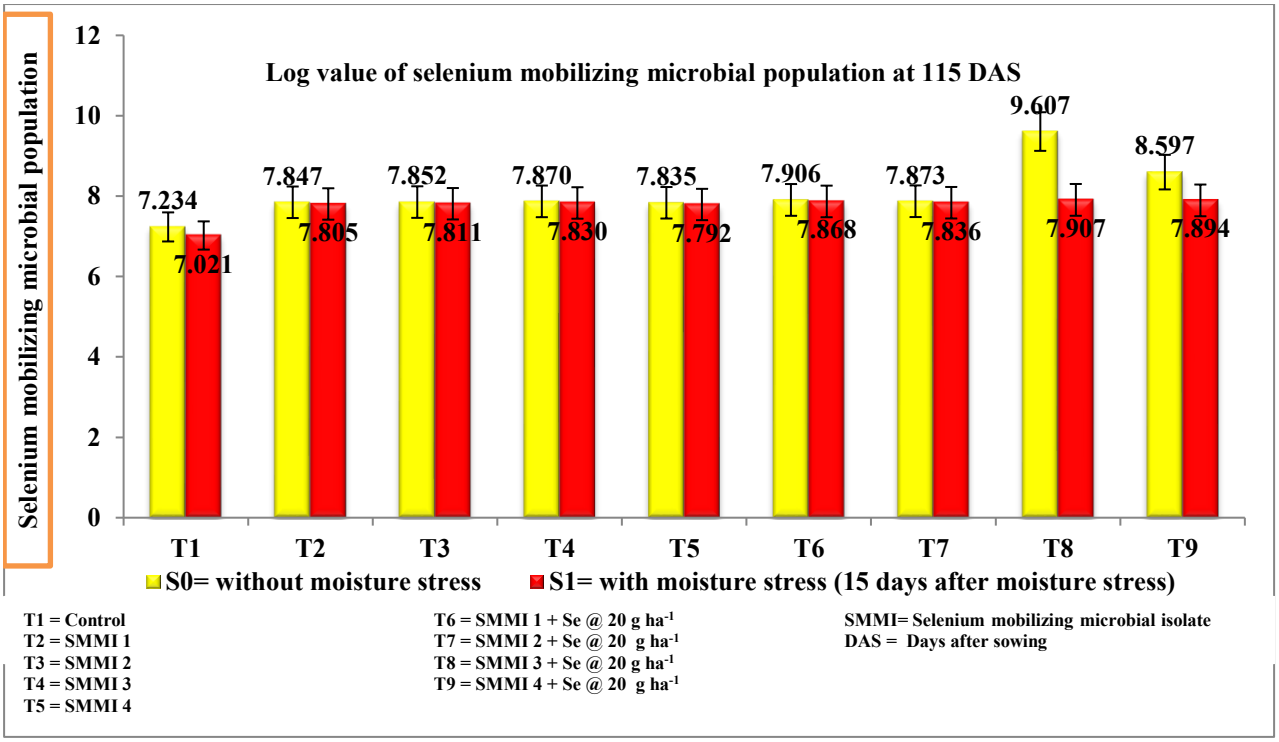


Fig.1 Effect of microbial isolates with and without Selenium on selenium mobilizing microbial population after inducing stress

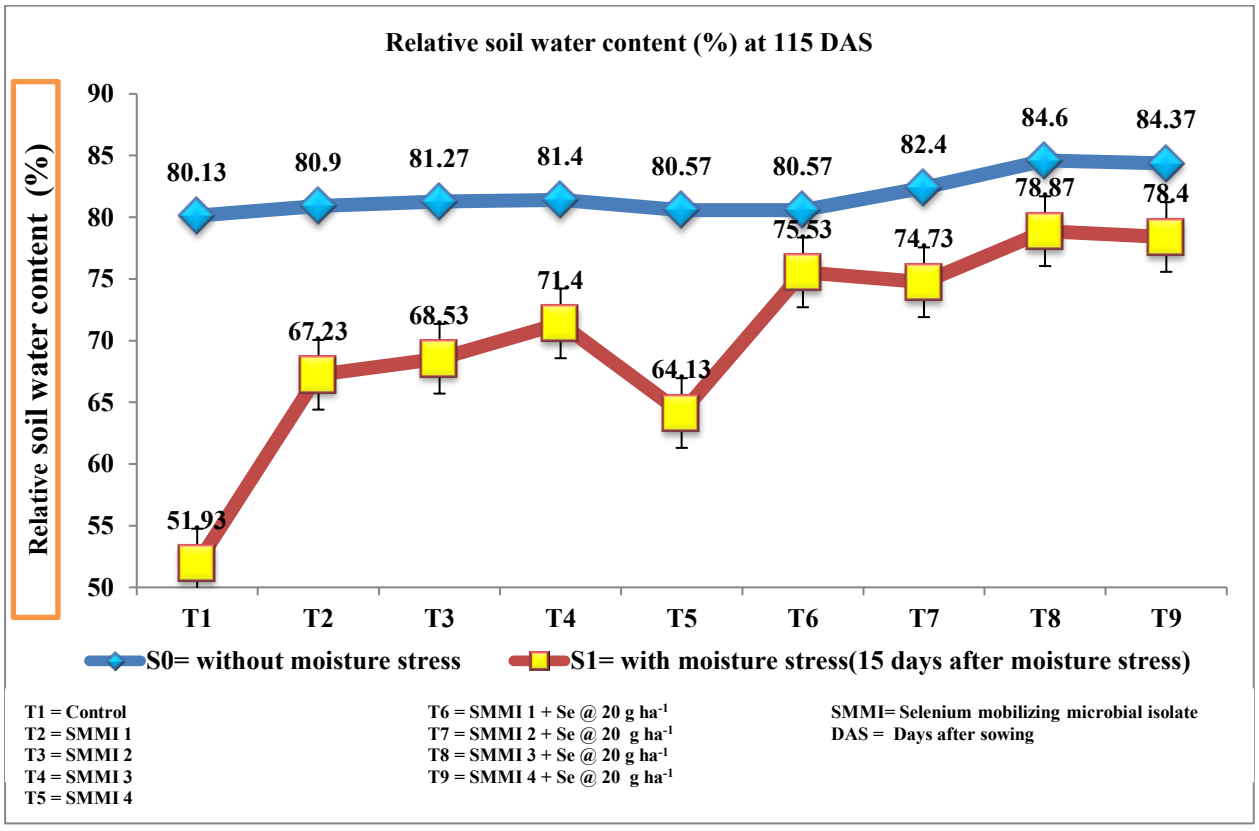


Fig. 2 Effect of microbial isolates with and without Selenium on Relative soil water content (%)

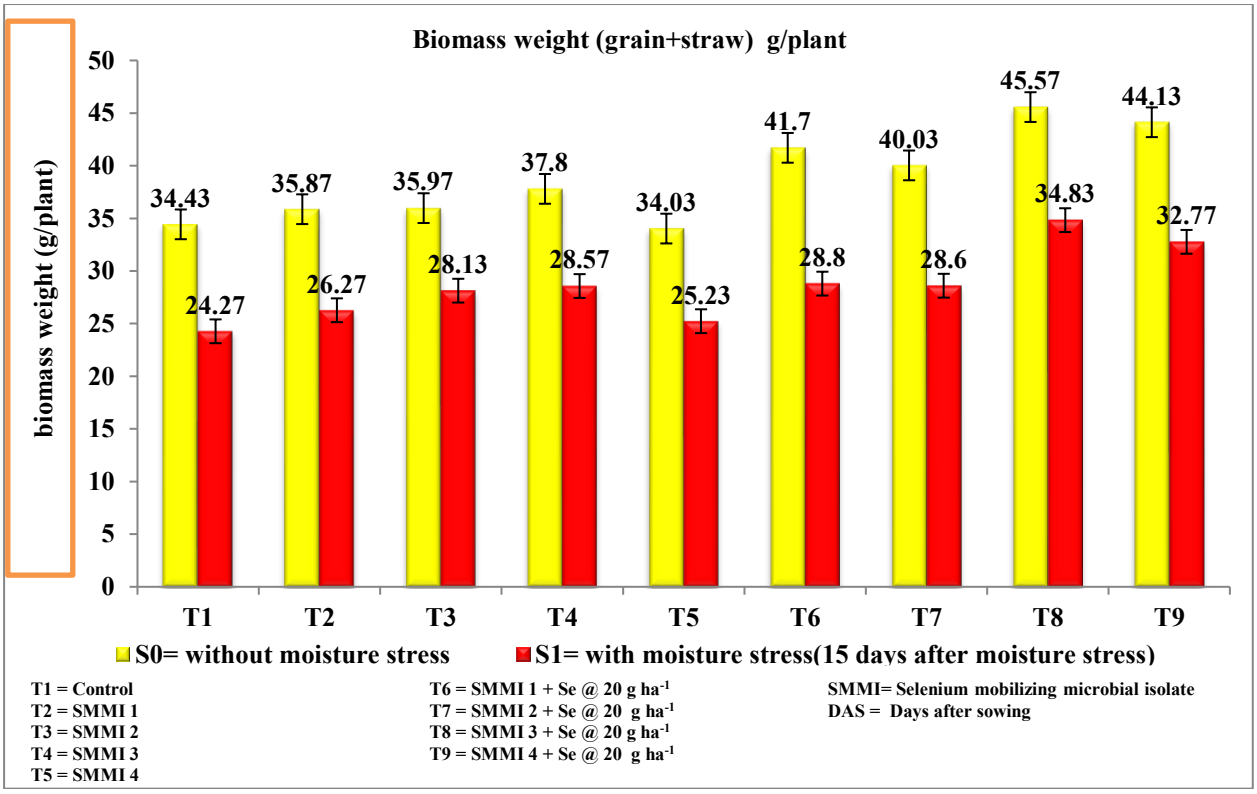


Fig. 3 Effect of microbial isolates with and without Selenium on Biomass weight (g/plant) of rice

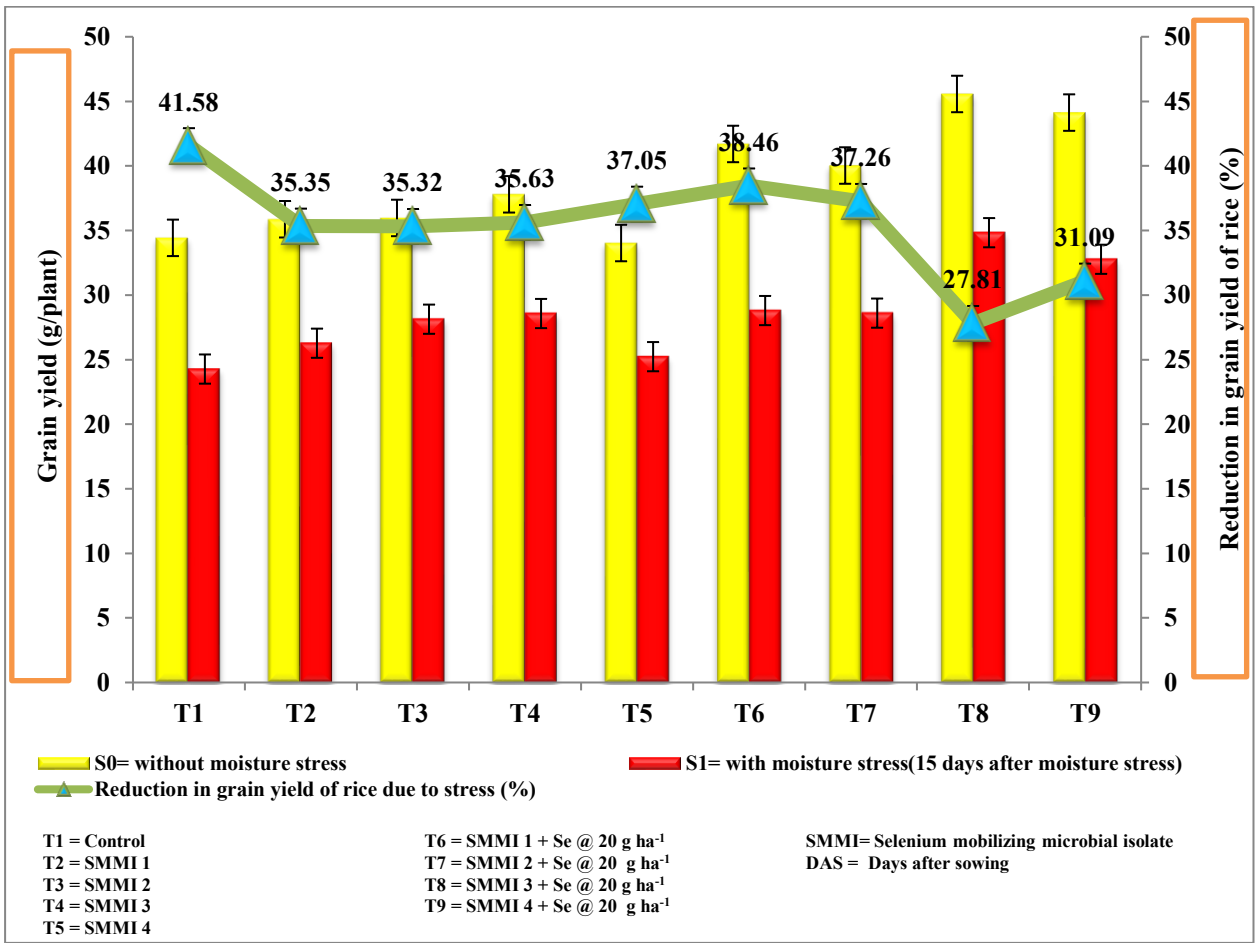
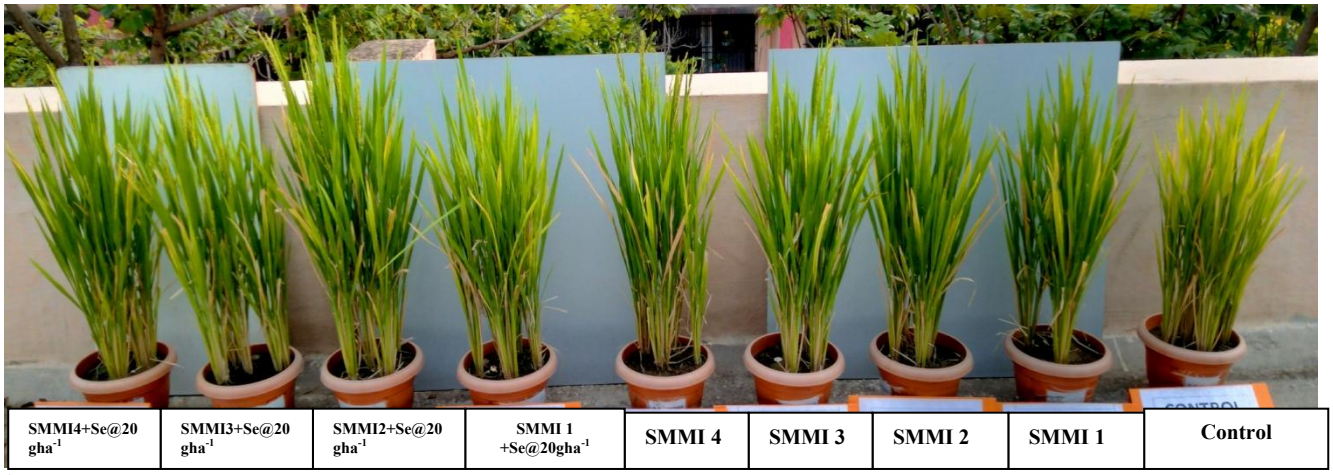
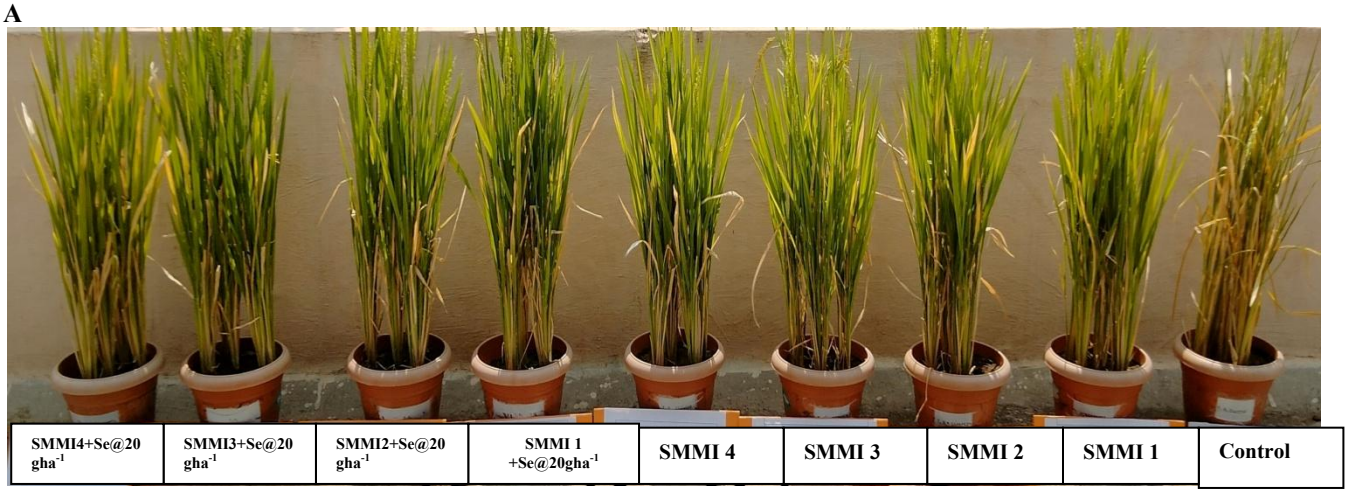


Fig. 4 Effect of microbial isolates with and without selenium on grain yield (g plant^{-1}) and reduction in grain yield (%) of rice.



A



B

Fig. 5 Photograph showing different treatment groups and the uninoculated control plants 120 days post-germination. Here, A- non-stressed, and B- stressed plants

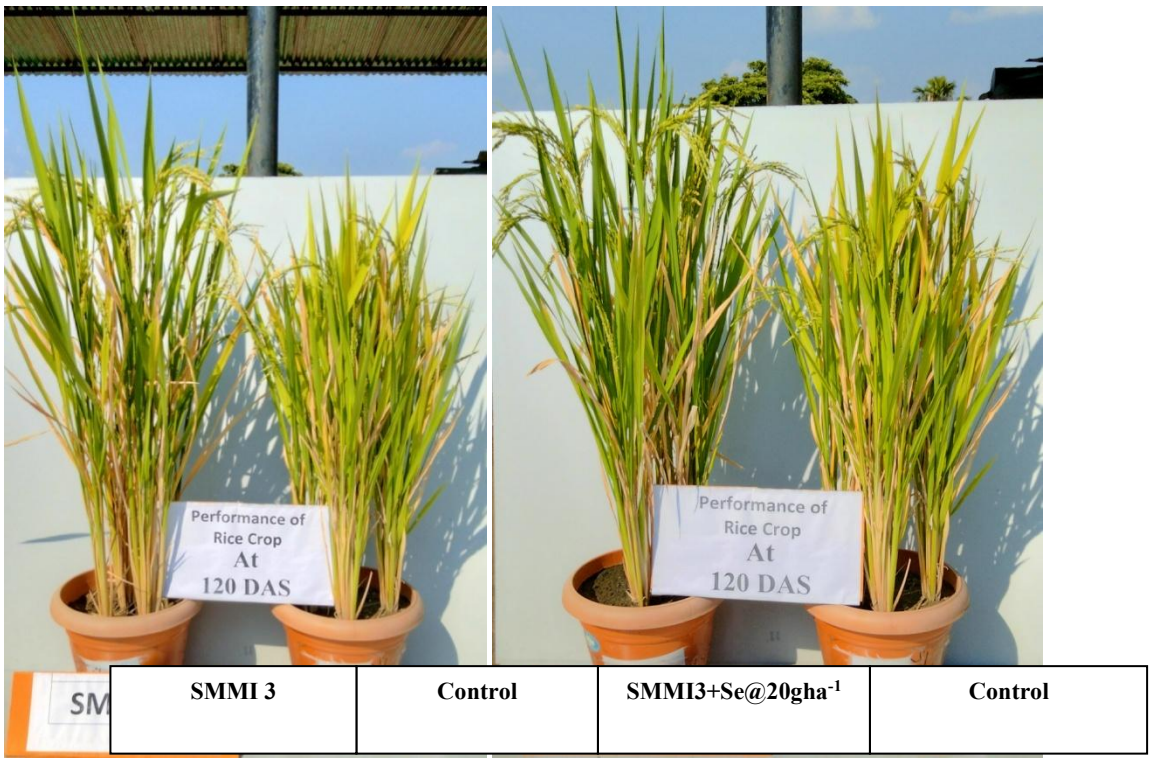


Fig. 6 Photograph showing performance of rice plants treated with SMMI-3 (Selenium mobizing microbial isolate-3; Bacillus sp. GD3b) with & without selenium (20g ha-1)

The results of the pot experiment revealed that, the population of selenium mobilizing microbial isolates (SMMI) was recorded at 30, 60, 90 and 115 days after sowing (DAS) (Figure 1) under non-stress (S_0) and stress (S_1) conditions. Moisture stress was imposed from 91 to 115 DAS; therefore, both treatments exhibited almost identical microbial population at 30, 60 and 90 DAS. At 30 DAS, the population ranged from 7.099 to 8.481(log value) under S_0 and 7.102 to 8.491 under S_1, where the maximum population was observed in T_8 (8.48–8.49) and the minimum in T_1 (7.09–7.10). A similar trend was observed at 60 DAS, where the microbial population varied from 7.219 to 8.500 under S_0 and 7.234 to 8.478 under S_1. At 90 DAS, the log population further increased and ranged from 7.356 to 8.636 under S_0 and 7.365 to 8.637 under S_1, indicating progressive microbial multiplication with crop growth under adequate soil moisture conditions. However, after the imposition of moisture stress, noticeable reductions in microbial population were observed at 115 DAS. The population ranged from 7.234 to 9.607(log value) under S_0, whereas it ranged from 7.021 to 7.907 under S_1. The highest microbial population was recorded in T_8 under S_0 (9.607), while the lowest value was observed in T_1 under S_1 (7.021). Moisture stress caused a reduction in microbial population ranging from 0.47% to 17.69% across treatments. The highest reduction (17.69%) was recorded in T_8, followed by 8.18% in T_9, whereas the lowest reduction (0.47–0.55%) was observed in treatments T_2 to T_7. On an overall mean basis, the microbial population decreased from 8.07 (S_0) to 7.75 (S_1), indicating an average reduction of about 3.93% due to moisture stress. Under treatment of T_8 (SMMI 3 + Se @ 20g ha-1) the overall population of selenium mobilizing microbial isolate was found maximum in stress condition which indicated its ability to survive under high moisture stress condition in presence of selenium.

The relative soil water content (RSWC) at 115 days after sowing (DAS) was markedly influenced by moisture stress and selenium mobilizing microbial isolates (SMMI), (Figure 2,5&6). Under non-stress conditions (S_0), soil water content ranged from 80.13 to 84.60%, whereas under moisture stress (S_1) it decreased to 51.93 to 78.87%, indicating a substantial decline due to stress imposition. Among the treatments, T_8 (SMMI 3 + Se @ 20 g ha^{-1}) recorded the highest soil water content under both conditions (84.60% in S_0 and 78.87% in S_1) with only 6.77% reduction due to moisture stress. Overall, moisture stress significantly reduced soil water content; however, inoculation with selenium mobilizing microbial isolates, particularly in combination with selenium application, helped maintain relatively higher soil moisture under stress conditions. This may be attributed to improved soil microbial activity and better soil aggregation and water retention in the rhizosphere, which ultimately mitigated the adverse effects of moisture stress. A study done by Nivedita et al. (2024) reported that a consortium of salt-resistant Streptomyces strains improved rice growth under abiotic stresses, including drought, by enhancing photosynthesis, antioxidant activity, and proline accumulation. These physiological enhancements are similar to the improved soil moisture retention and growth responses observed in microbial treatments combined with selenium application.

Moisture stress markedly influenced the dry biomass (grain + straw) of plants. Dry weight (grain + straw) decreased considerably due to moisture stress (Figure 3). Under S_0, dry weight varied from 34.03 to 45.57 g $plant^{-1}$, which declined to 24.27 to 34.83 g $plant^{-1}$ under S_1. The highest dry weight was recorded in T_8 (45.57 g $plant^{-1}$ in S_0 and 34.83 g $plant^{-1}$ in S_1). Moisture stress resulted in 23.57% to 29.51% reduction in dry weight across treatments. Recent studies have shown that inoculation with beneficial endophytic bacteria significantly enhances rice growth and drought tolerance, resulting in improved plant biomass and stress resilience under moisture deficit conditions (Kashyap et al.,2025).

Moisture stress (S_1) also reduced grain and straw yield of rice significantly compared to non-stress conditions (S_0). Grain yield under S_0 ranged from 17.17 to 22.90 g $plant^{-1}$, which declined to 10.03–16.53 g $plant^{-1}$ under stress (Figure 4). The highest grain yield was recorded in T_8 (SMMI 3 + Se @ 20 g ha^{-1}) with 22.90 g $plant^{-1}$ in S_0 and 16.53 g $plant^{-1}$ in S_1, corresponding to a 27.81% reduction, whereas the control (T_1) showed the highest reduction (41.58%). Application of SMMI along with selenium reduced yield losses, with grain yield saving ranging from 3.12 to 13.77%, highest in T_8 and lowest in T_6, indicating that microbial inoculation with selenium partially mitigated the adverse effects of moisture stress. Patnaik et al. (2023) reported that drought stress significantly reduced rice grain and straw yield, and foliar application of selenium improved these traits under stress by enhancing physiological performance, showcasing typical grain and straw response under moisture deficit conditions.

The most effective selenium mobilizing isolate (SMMI-3) was identified as Bacillus subtilis strain GD3b by 16S rRNA gene sequence using Sanger sequencing (BDT v3.1 Cycle Sequencing Kit) study, was found to mitigate abiotic stress in the rice crop in the study. Similar results were reported by de Lima et al. (2019), who demonstrated that Bacillus subtilis ameliorates water stress tolerance in maize and common bean. Their study showed that B. subtilis promoted plant growth and enhanced water use efficiency in both crops. Inoculation with B. subtilis increased leaf water content and improved stomatal regulation without adversely affecting photosynthetic rates. Furthermore, antioxidant activity in both plants was reduced following bacterial inoculation, indicating alleviation of stress conditions. Overall, their findings suggest that B. subtilis can be effectively used as a bioinoculant to protect maize and common bean against water stress. Similar results were reported by Thampi et al. (2022), who highlighted the critical role of endophytic Bacillus spp. in promoting plant health and enhancing tolerance to abiotic stress in rice. Their study emphasized that bacterial endophytes function as plant growth–promoting agents by producing phytohormones and facilitating plant growth in contaminated soils through the degradation of hazardous compounds. These endophytes help regulate plant growth under adverse environmental conditions such as salinity, drought, temperature extremes, heavy metal toxicity, and nutrient deficiency through multiple physiological and

biochemical mechanisms.

The study demonstrates that selenium-rich soils harbor diverse microbial populations capable of supporting crop growth under stress. Among the isolates, Bacillus subtilis strain GD3b effectively mitigated moisture stress in rice, maintaining higher microbial populations, soil moisture, plant biomass, and grain/straw yields. Inoculation with selenium mobilizing microbes (SMMI) combined with selenium, particularly T_8 (SMMI 3 + Se @ 20 g ha^{-1}), enhanced plant stress tolerance and productivity. These findings highlight the potential of using SMMI as a bioinoculant for improving rice resilience under water-limited conditions, warranting further field-level validation to optimize its practical application.

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