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The Prostrate Spurge-isolated PGPB Endophytes, EP1-AS, and EP1-BM That Can Tolerate High Levels of Salinity and Heavy Metals and Allow Wheat Growth Under These Stressors

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ABSTRACT

This research investigates the potential of two Plant Growth-Promoting Bacteria (PGPB) strains, EP1-AS and EP1-BM, isolated from the halophyte *Euphorbia prostrata*, to enhance plant growth and provide abiotic stress resilience. The study addresses the urgent need for sustainable agricultural practices in the face of challenges like soil salinization and heavy metal contamination. The investigation comprehensively analyzes the heavy metal and salt tolerance of the PGPB strains, revealing their potential applications in promoting plant growth under adverse environmental conditions. The research further explores the impact of these PGPB strains on wheat plants subjected to varying concentrations of heavy metals and salts. Results indicate that both PGPB strains, especially EP1-BM, exhibit significant tolerance to heavy metals and salt stress. EP1-BM demonstrates remarkable resilience even under high concentrations of these stressors. The study extends its findings to in vitro testing on wheat plants, revealing the positive influence of PGPB strains on germination, shoot length, and root length in the presence of salt and heavy metals. This research underscores the significance of understanding plant-microbe interactions, particularly in the context of promoting sustainable agriculture in challenging environments. The identified resilience of PGPB strains, especially EP1-BM, suggests their potential application as bio-remediators and plant growth promoters in soils affected by salinity and heavy metal stress. The promising results observed will be followed-up field trials. They will highlight the translational potential of these PGPB strains, offering a novel avenue for developing biofertilizer formulations with a cautious approach to safety concerns. Overall, this study contributes valuable insights into harnessing the untapped potential of resilient plants and their associated microbial communities for sustainable agriculture. It addresses key global challenges outlined by the United Nations Sustainable Development Goals.

INTRODUCTION

A confluence of environmental challenges, encompassing pollution, the impact of coronaviruses, and the far-reaching consequences of climate change, has compounded recent global economic meltdowns. Among the environmental stressors, pollution from heavy metals and salinity has emerged as a substantial threat, exerting diverse and complex pressures on economic stability. Heavy metal pollution, often stemming from industrial processes, not only disrupts ecosystems but also imposes significant economic burdens (Vinayak 2020a, 2020b, Sharma 2021, Mudgal et al. 2021). Contaminated water sources, soil degradation, and compromised agricultural productivity lead to escalating cleanup costs, increased healthcare expenditures, and disruptions in global food supply chains. Industries reliant on pristine water resources encounter heightened operational challenges, intensifying economic strain. Salinity, exacerbated by climate change and suboptimal agricultural practices, presents an additional economic menace. Soil salinization negatively impacts crop yields, contributing to reduced agricultural output and escalating food prices.

Moreover, salinity-induced deterioration of infrastructure, especially in coastal regions, entails increased maintenance costs and undermines the durability of structures. Adding to the economic complexity, the ongoing challenges posed by coronaviruses have disrupted global trade, supply chains, and labor markets (Kaur & Mudgal 2021). This pandemic has underscored the intricate interplay between economic vulnerabilities and environmental stressors, emphasizing the imperative of comprehensive strategies to address concurrent threats. Climate change, characterized by an uptick in extreme weather events, further amplifies economic risks worldwide. Rising sea levels, more intense storms, and shifting precipitation patterns contribute to increased infrastructure damage, surging insurance claims, and challenges for industries reliant on climate-sensitive resources. Amidst these challenges, biotechnological initiatives have emerged as promising avenues for relief. Innovations in biotechnology offer potential solutions for remediating heavy metal pollution, improving soil salinity tolerance in crops, and even contributing to medical advancements for pandemic preparedness (Kaur & Mudgal 2021). Harnessing biotechnological tools allows for targeted interventions, fostering resilience and sustainable practices in the face of multifaceted economic and environmental threats. The escalating threats of soil salinization, heavy metal contamination, and other environmental challenges pose a substantial risk to agricultural productivity, thereby imperiling global food security. Conventional agricultural methodologies often prove inadequate in mitigating these challenges, necessitating the exploration of sustainable and ecologically sound alternatives. Recent years have witnessed a burgeoning interest in harnessing the potential of Plant Growth-Promoting Bacteria (PGPB) to augment plant growth and confer resistance against abiotic stressors.

Spurges, belonging to the expansive Euphorbiaceae family of flowering plants, exhibit a global distribution and are distinguished by their milky sap and diverse morphologies (Webster 1967). With numerous species employed in traditional medicine and some extensively scrutinized for their biological and biochemical attributes (Pascal 2017), spurges have recently garnered increased significance in the realms of biology and biotechnology. Their potential applications span diverse fields, encompassing medicine, agriculture, and industry. Extensive studies on the biology of spurges have unveiled a repertoire of bioactive molecules, including alkaloids, flavonoids, terpenoids, and phenolic compounds (Akdad et al. 2022). These compounds exhibit multifaceted biological activities such as anti-inflammatory, anti-oxidant, and anti-cancer properties (Singh 2021). Notably, certain spurges have been investigated for their prowess in bioremediation, contributing to environmental sustainability (Jeevanandam 2021). The field of spurge biotechnology has witnessed substantial progress, with genetic engineering techniques harnessed to modify plants for bioremediation purposes. Certain spurges also serve as valuable models for delving deeper into plant biology and biochemistry (Chan, Crabtree et al. 2010). For instance, *Euphorbia lathyris* has been instrumental in studying plant hormones (Sivakumaran & Hall 1978, Mingo-Castel 1984), while *Euphorbia milii* has contributed to the understanding of plant stress responses (Khaksar et al. 2016). Beyond their role in bioremediation, spurges have exhibited potential applications in medicine. Some species display anti-cancer and anti-inflammatory activities, aligning with their traditional medicinal use for various ailments (Salatino et al. 2007).

Moreover, spurges have attracted attention for their potential role in biofuel production, with certain species possessing high triglyceride levels suitable as an energy source (Linfang et al. 2021, Srivastava & Soni 2019). The dynamic landscape of spurge biology and biotechnology underscores the breadth of their potential applications across various domains. Future research endeavors should delve deeper into understanding the molecular mechanisms of these plants to unlock their full potential and further elucidate their applications in diverse fields.

Our work culture themes over exploring various hardy, succulent and resilient plants and their interaction with the microbial communities and anticipating the research outcomes to translate into enhancement of crop productivity under various stressors (Ramamoorthy et al. 2009, Siva et al. 2009, Mudgal & Mudgal 2011, Mudgal et al. 2011, Kaur & Mudgal 2020a, 2020b, Parashar 2020a, 2020b, Vinayak 2020, 2021, Kaur & Mudgal 2021, Sharma 2021, Mudgal et al. 2021, 2011, Kaur et al. 2022, 2023a, 2023b, Parashar et al. 2023, Saeed et al. 2023).

Of the many Euphorbias, *Euphorbia prostrata*, a halophyte plant renowned for its robustness in adverse environmental conditions, has become a focal point of investigation (Webster 1967, Dubyna et al. 2022, Unde & Kumar 2022). The ramifications of salinity and heavy metal pollution on plant growth are profound, impacting nutrient uptake, water balance, cellular homeostasis, and various physiological processes. PGPB emerges as a promising solution to alleviate these stressors, leveraging mechanisms such as phytohormone production, nutrient solubilization, and the regulation of stress-responsive genes. Expanding our understanding of the distinctive traits exhibited by PGPB isolates derived from *Euphorbia prostrata* holds immense potential. This insight can serve as a foundation for evaluating their efficacy as biotechnological tools, offering solutions to bolster sustainable agriculture in challenging environmental conditions. The exploration of PGPB isolates, in conjunction with the resilience showcased by halophytes like *Euphorbia prostrata*, represents a strategic approach to advancing agricultural practices that align with environmental sustainability and global food security imperatives.

In a preceding investigation (Parashar et al. 2023), we successfully isolated two co-culturable bacterial endophytes, EP1-AS and EP1-BM, originating from the stem internodal segments of *Euphorbia prostrata*, a succulent member of the Euphorbiaceae family. Employing a comprehensive approach involving morphological, biochemical, and molecular analyses, we identified these strains as novel members of Enterobacteriaceae, specifically *Lelliotia amnigena*. Both isolates exhibited substantial plant growth promotion (PGP) potentials during rigorous assays. EP1-BM manifested as swiftly expanding swarms, while EP1-AS exhibited growth in the form of rounded colonies on nutrient agar. The PGP effects of these isolates were substantiated through meticulous in vitro and ex-vitro seed-priming treatments with wheat and tomato seeds. Remarkably, these treatments resulted in significantly elevated seed germination rates and noteworthy enhancements in morphometric and physiological aspects of plant growth. The validation extended into field trials, where both EP1-AS and EP1-BM demonstrated commendable yields in wheat grain and tomato fruit harvests. This groundbreaking study represents the pioneering exploration of PGPB endophytes within *Euphorbia prostrata*, shedding light on their potential contributions to enhancing agricultural productivity. The results underscore the significance of these novel bacterial strains in promoting sustainable plant growth and heralding a promising avenue in the context of plant-microbe interactions and agroecological practices.

The present study seeks to explore the potential of the above two PGPB endophytes isolated from a sandmat spurge in enhancing plant survival and physiological growth under the effect of salinity and exposure to various heavy metals.

MATERIALS AND METHODS

Culturing Bacterial Endophytes EP1-AS and EP1-BM

Two PGPB endophytic bacteria, EP1-AS and EP1-BM, were isolated from *Euphorbia prostrata* and characterized for their PGP profiles previously (Parashar et al. 2023). Each was revived from a glycerol stock to raise pure colonies over ampicillin-supplemented nutrient agar (NA) plates with conditions specified previously. From a single colony for each, a 17 hrs old pure culture with an overnight cell density of ≥ 10^9 CFU/ml was inoculated in 100ml of Nutrient Broth (NB) and incubated, was incubated at 37°C with orbital shaking at 200 rpm overnight. For a control, *Escherichia coli* was used at a similar inoculum size. All chemicals and reagents were of research grade (Himedia, Mumbai, India).

Measuring Heavy Metals and Salt Tolerance of EP1-AS and EP1-BM

The susceptibility of bacterial strains to four distinct heavy metal salts viz., sodium arsenite ($NaAsO_2$), lead nitrate [$Pb(NO_3)_2$], chromium trioxide (CrO_3), cadmium sulfate ($CdSO_4$) was evaluated in accordance with Washington's (1980) agar dilution methodology (Washington 1980). Stock solutions of the following heavy metal salts were prepped and filtered through a 0.22 µm Millipore filter to guarantee sterility. For each heavy metal concentration (200 ppm, 500 ppm, 700 ppm, and 1000 ppm), respectively, each salt was added to the autoclaved 30 mL of NB agar medium and incubated at a temperature of 37°C. Likewise, to create salt stress, stock solutions of different salt concentrations (1%, 2%, 3%, 4%, and 5%) were prepared and filtered through the same Millipore filter as used in the former. Later, the stocks were added to an autoclaved NB medium at a temperature of 37°C. Visual observations were scored based on the growth of new streaks. For a control, Escherichia coli was used at a similar inoculum size. All chemicals and reagents were of research grade (Himedia, Mumbai, India).

Surface Sterilization and Seed Priming of Wheat Seeds

Wheat seeds of a variety named UNNAT PBW 343 were procured from a local shop in Mohali Punjab) and were surface sterilized with 70% ethanol and flash treatment of mercuric chloride for 10 seconds, along with three later washes of sterile distilled water. Surface sterilized 20 wheat seeds each for EP1-AS, EP1-BM, and control sets were primed with 17 hrs old fresh inoculum (≥ 10^9 CFU.mL^{-1}) of EP1-AS and EP1-BM prepared in NB media and were kept in overnight incubation. For control sets, the seeds were put overnight only in sterile NB media.

Preparation of Setup for Experiment

For the experimental setup, 15 EP1-AS, EP1-BM primed seeds, and unprimed control seeds were placed on tissue paper wetted with the 15 mL of desired concentration of heavy metals and salt and placed on a plastic petri dish. 15 mL of prepped stocks for each concentration were poured onto tissue paper containing prepped seeds. Separate independent trials were run each for salt and heavy metal stress treatments according to the desired concentration (ppm) applied every 5 days. Observations were recorded every 2 days for germination, root, and shoot length. The control treatment sets were avoided with seed treatments with endophytes, but the same was replaced with plain NB.

Statistical Analyses and Data Presentation

All experiments were done thrice with three or more replicates per trial for each variable. Data in graphs showed the average of the or more trials with averaged replicates, and standard errors were depicted with error bars inferring standard deviations from the mean values. Pictures in the figures were representative seedlings for the observed trends in each of the treatment trials shown with a scale bar for clarity and measurable distinguishments. Graphs were prepared in MS Excel, along with pictures in figures assembled and adjusted using MS PowerPoint.

RESULTS AND DISCUSSION

The EP1-AS and EP1-BM Isolates

Both the novel *L. amnigena* isolates were previously characterized at our end (Parashar et al. 2023). In brief, as before, the isolates showed up as peculiar off-white bacterial growth when revived over nutrient agar (NA) plates with ampicillin. These bacteria were later tested under treatment in liquid suspension media with various stressors.

EP1-AS and EP1-BM Tolerating High Salt and Heavy Metal Levels in Culture Media

Salinity tolerance of EPI-AS and EP1-BM: Table 1 shows the effect of various salt concentrations on the growth of the two bacterial isolates. Upon elevating the salt concentration to 2%, both the control and EP1-AS strains exhibited mild growth, whereas EP1-BM demonstrated robust growth, indicating its exceptional tolerance to elevated salt levels. At 3% salt concentration, the control and EP1-AS maintained moderate growth, while EP1-BM sustained vigorous growth, underscoring its remarkable ability to thrive in highly saline environments. Upon further escalation to 4% salt concentration, the control exhibited no growth, signifying its sensitivity to heightened salinity. In contrast, both EP1-AS and EP1-BM strains displayed moderate growth, indicating their capacity to endure this salt concentration. Notably, at a 5% salt concentration, neither the control nor EP1-AS strains exhibited any growth, revealing their susceptibility to elevated salinity. In stark contrast, EP1-BM exhibited exceptional growth, showcasing its outstanding salt tolerance, even in the face of heightened salinity. These findings underscore the significance of EP1-BM as a potential candidate for enhancing salt tolerance in crops subjected to saline conditions. The robust growth exhibited by EP1-BM, particularly at higher salt concentrations, positions it as a promising microbial ally in mitigating the adverse effects of salinity on plant growth.

Further exploration into the molecular mechanisms underlying EP1-BM's exceptional salt tolerance could unveil valuable insights for developing biotechnological interventions aimed at improving crop resilience in saline environments. The identified differential responses among EP1-AS, EP1-BM, and the control also emphasize the intricate interplay between endophytic bacteria and plant host EP in coping with environmental stressors. These insights pave the way for future investigations into harnessing the potential of endophytes, such as EP1-BM, for sustainable agricultural practices in salt-affected regions.

Table 1: Two PGPB endophyte strains (EP1-AS and EP1-BM) and a control bacterium (*Escherichia coli*) were tested at various salt concentrations (1% to 5%). The bacterial growth response was scored as follows: "++++": abundant growth; "+++": moderate growth; "++" lower than moderate" and "-": no visible growth.

	Salinity (%)				
	1	2	3	4	5
Control	++	+	+	-	-
EP1-AS	++++	+++	+++	++	-
EP1-BM	++++	++++	++++	+++	++

Heavy metals tolerance of bacterial endophytes: Table 2 summarizes the effect of all the heavy metals on the growth of the two EP endophytes seen in plate assays:

***Sodium arsenite ($NaAsO_2$)*:** At 200 ppm of sodium arsenite, both the control and AS strains displayed moderate growth, signifying their tolerance to the elevated concentration. However, EP1-BM exhibited remarkable growth (++), showcasing its resilience even at this lower concentration. Upon increasing the concentration to 500 ppm, EP1-AS and the control both exhibited moderate growth (+), whereas EP1-BM displayed significant growth (++), highlighting its exceptional tolerance to higher concentrations. At 1000 ppm of sodium, both the control and EP1-AS strains showed no growth (-), indicating their sensitivity to extreme levels of the heavy metal. In contrast, EP1-BM continued to exhibit remarkable growth (++), demonstrating its remarkable ability to withstand even the highest concentrations of sodium arsenite.

***Lead nitrate [$Pb(NO_3)_2$]*:** At a concentration of 200 ppm of lead nitrate, the control displayed moderate growth (+), indicating its tolerance to the high concentration of the heavy metal. Both EP1-AS and EP1-BM strains exhibited remarkable growth (+++), showcasing their resistance to lead nitrate. Upon increasing the concentration to 500 ppm, the control and EP1-AS strains both exhibited moderate growth (+), indicating their tolerance to higher levels of lead nitrate. EP1-BM also showed abundant growth (+), demonstrating its resilience to higher concentrations of lead. At 700 ppm, the control did not show any growth (-), suggesting its susceptibility to the high concentration. At 1000 ppm, both EP1-AS and EP1-BM strains exhibited growth (+), demonstrating their tolerance to even higher levels of lead nitrate.

***Chromium trioxide (CrO_3)*:** The control was sensitive to chromium trioxide and failed to survive at concentrations ranging from 200 ppm to 1000 ppm. EP1-AS tolerated concentrations up to 500 ppm, displaying visible growth, while EP1-BM tolerated up to 1000 ppm, showing remarkably visible growth.

***Cadmium sulphate ($CdSO_4$)*:** The control was non-responsive to all cadmium sulfate concentrations ranging from 200 to 1000 ppm. In contrast, EP1-AS tolerated up to 700 ppm of this heavy metal, and EP1-BM showed visible growth even at 1000 ppm.

In summary, the results reveal distinct levels of salt and heavy metal tolerance among the bacterial strains. While the control demonstrated sensitivity to increasing concentrations, both EP1-AS and EP1-BM displayed robust resistance to higher salinity and heavy metal stress, with EP1-BM emerging as the more resistant strain. This suggests that EP1-BM holds potential as a bio-remediator and agronomist in environments with salinity and heavy metal stress. This invites further exploration into EP1-BM's stress response mechanisms and its role as a plant growth promoter in salt-damaged agricultural soils. Moreover, the specific tolerance exhibited by EP1-BM to lead, chromium, and cadmium positions it as a promising candidate for biotechnological applications aimed at mitigating heavy metal contamination in crops and soils.

Table 2: Two PGPB endophyte strains (EP1-AS and EP1-BM) and a control bacteria (*Escherichia coli*) were tested at various heavy metal concentrations in plate assay, inferring growth as streaks. The bacterial growth response was scored as follows: "+++": abundant growth; "++": moderate growth; and "-": no visible growth.

	Concentrations (ppm)			
	200	**500**	**700**	**1000**
Sodium arsenite				
Control	+	+	-	-
EP1-AS	++	++	+	+
EP1-BM	+++	+++	++	+
Lead nitrate				
Control	++	+	-	-
EP1-AS	+++	++	++	+
EP1-BM	+++	++	++	++
Chromium trioxide				
Control	-	-	-	-
EP1-AS	+	+	-	-
EP1-BM	++	+	+	+
Cadmium Sulphate				
Control	-	-	-	-
EP1-AS	+	+	+	-
EP1-BM	+	+	+	+

EP1-AS and EP1-BM Priming Restores Wheat Seedling Growth Under High Salinity

The PGPB endophytes isolated from EP displayed a remarkable and promising impact on wheat seed treatments over time, particularly when compared to untreated controls. This emphasizes their potential plant growth-promoting (PGP) attributes, which align with the findings from previous biochemical PGP assays conducted in earlier research. In the current study, we examined the influence of salt on seeds treated/untreated with EP1-AS/EP1-BM in three distinct trials.

Untreated wheat seeds in the control group exhibited visibly compromised development as salt concentration increased (Fig. 1a), consistent with observations in the existing literature. However, priming seeds with either EP1-AS/EP1-BM endophytes significantly rescued seed germination, especially in the presence of 100 mM onwards up to 200 mM salt concentration, compared to control untreated seed lots (Fig. 1a). Notably, even at the highest salinity of 300 mM, BM maintained superior germinating seedling growth compared to AS, while controls without EP1-AS/EP1-BM treatment showed negligible propagules in any seed germination trials (Fig. 1a). Moreover, control plants demonstrated tolerance up to only 200 mM salinity with limited shoot growth, beyond which, at 250 and 300 mM, shoot growth was impeded (Fig. 1b). In contrast, EP1-AS and EP1-BM bacterized seeds exhibited increased tolerance to elevated salinity compared to control seeds. This tolerance is further supported by the maintenance of root length and vigor (Fig. 1c and 1d). Among the three, EP1-BM exhibited a stronger response in rescuing wheat seeds under higher salt stress compared to EP1-AS and the control. This suggests that the bacterization of wheat seeds with EP1-AS and, more prominently, with EP1-BM enhances tolerance against salinity.

From a perspective standpoint, these results underscore the potential application of EP1-BM as a valuable resource in enhancing crop resilience to salinity stress. EP1-BM, exhibiting a stronger response in rescuing wheat seeds under higher salt stress, holds promise for sustainable agriculture in saline environments. Further research avenues could explore the molecular mechanisms underlying the differential responses of EP1-AS and EP1-BM, providing insights into optimizing plant-endophyte interactions. Long-term investigations into the impacts of bacterization on crop productivity and soil health could contribute to more comprehensive and applicable outcomes in the field of agriculture, aligning with the global pursuit of sustainable development goals.

EP1-AS and EP1-BM Priming Restores Wheat Seedling Growth Under High Heavy Metal Concentrations

As for salinity stress, we tested the effect of EP1-AS/EP1-BM bacterization over wheat under stress from four selected heavy metal salts as well, namely, sodium arsenite, lead nitrate, cadmium sulfate, and chromium trioxide. In the case of sodium arsenite and lead nitrate (Fig. 2 and Fig. 3), the EP1-AS/EP1-BM untreated controls showed a drastic decrease in the emergence of propagules with time and in the overall number of seeds responding with germination as surmised from the effects at 200 ppm and

pursuit of sustainable development goals.

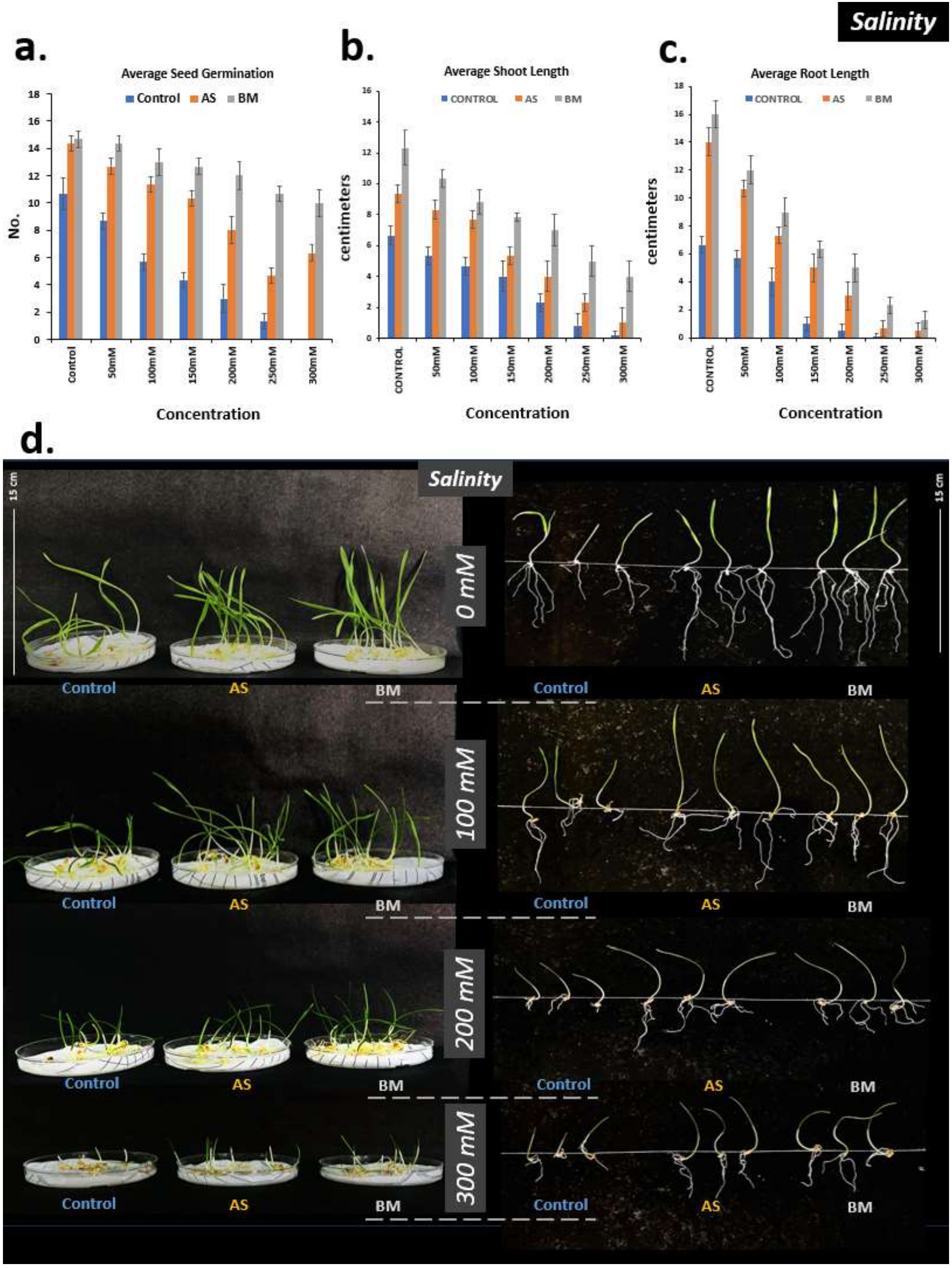

Fig. 1: Effect of seed bacterization treatments with EP1-AS and EP1-BM under various levels of salinity. Here, the parameters shown are **a)** average germination from 20 wheat seeds per replicate per trial, **b)** shoot length development, and **c)** root length development. The results represent the averaged outcomes of three independent trials. In **d),** scale bars represent the lengths of seedlings under different variables.

500 ppm of these heavy metals. However, lead nitrate seems more affecting with much higher detrimentally over wheat seedling emergence, which seemed almost abrogated under 700 ppm and 1000 ppm. Under seed treatments with EP1-AS/EP1-BM, however, germination was normalized and rescued at all levels of increased heavy metal concentrations (Fig. 2a and 3a). This rescue feature was always seen more significantly improved with BM-treated seed lots compared to EP1-AS treated lots. However, if we see the shoot growth statistics (Fig. 2b and 3b), the productive seedling growth

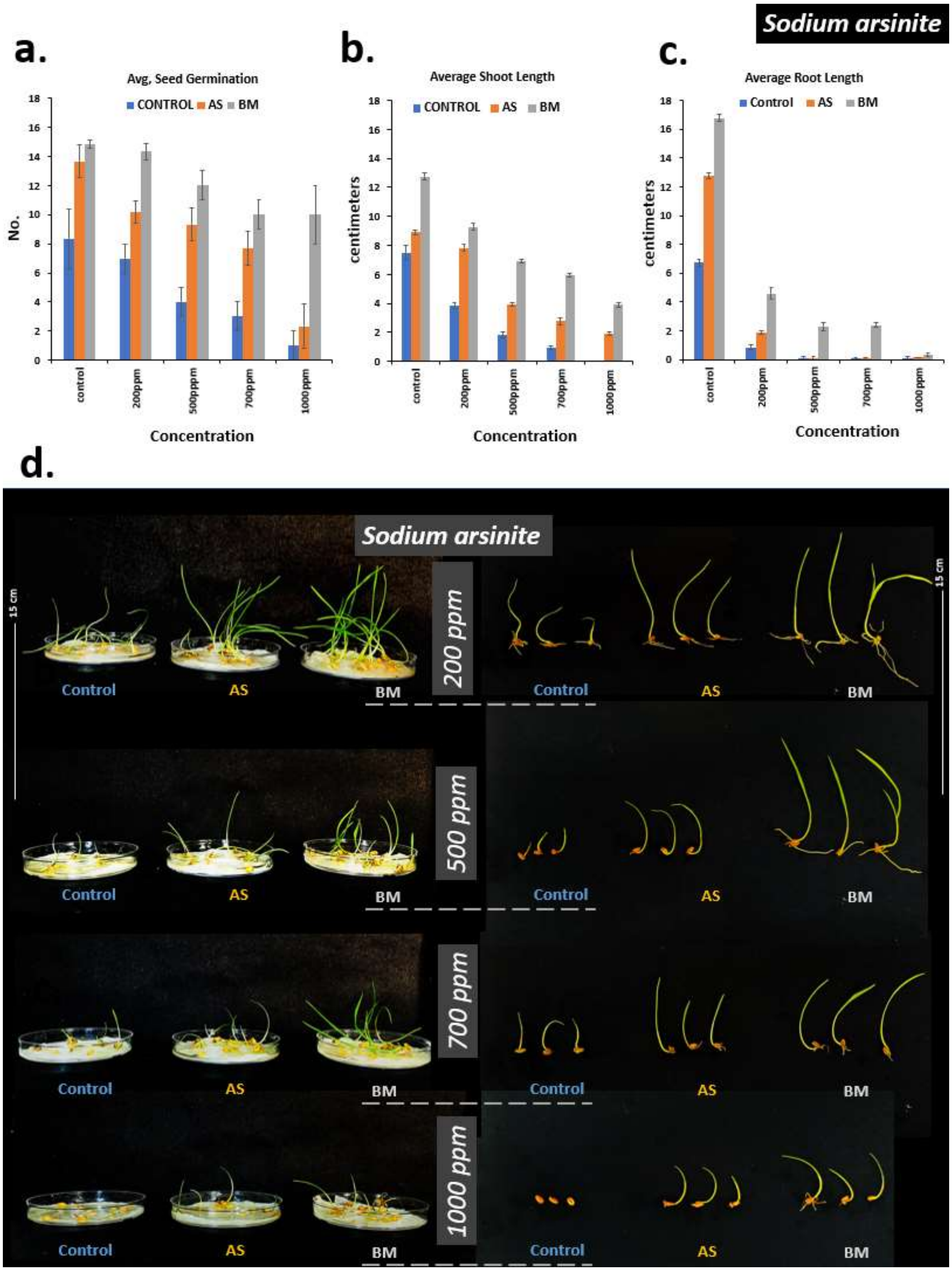

Fig. 2: Effect of seed bacterization treatments with EP1-AS and EP1-BM under various levels of the heavy metal sodium arsenite. Here, the parameters shown are a) average germination from 20 wheat seeds per replicate per trial, **b)** shoot length development, and **c)** root length development. The results represent the averaged outcomes of three independent trials. In **d),** scale bars represent the lengths of seedlings under different variables.

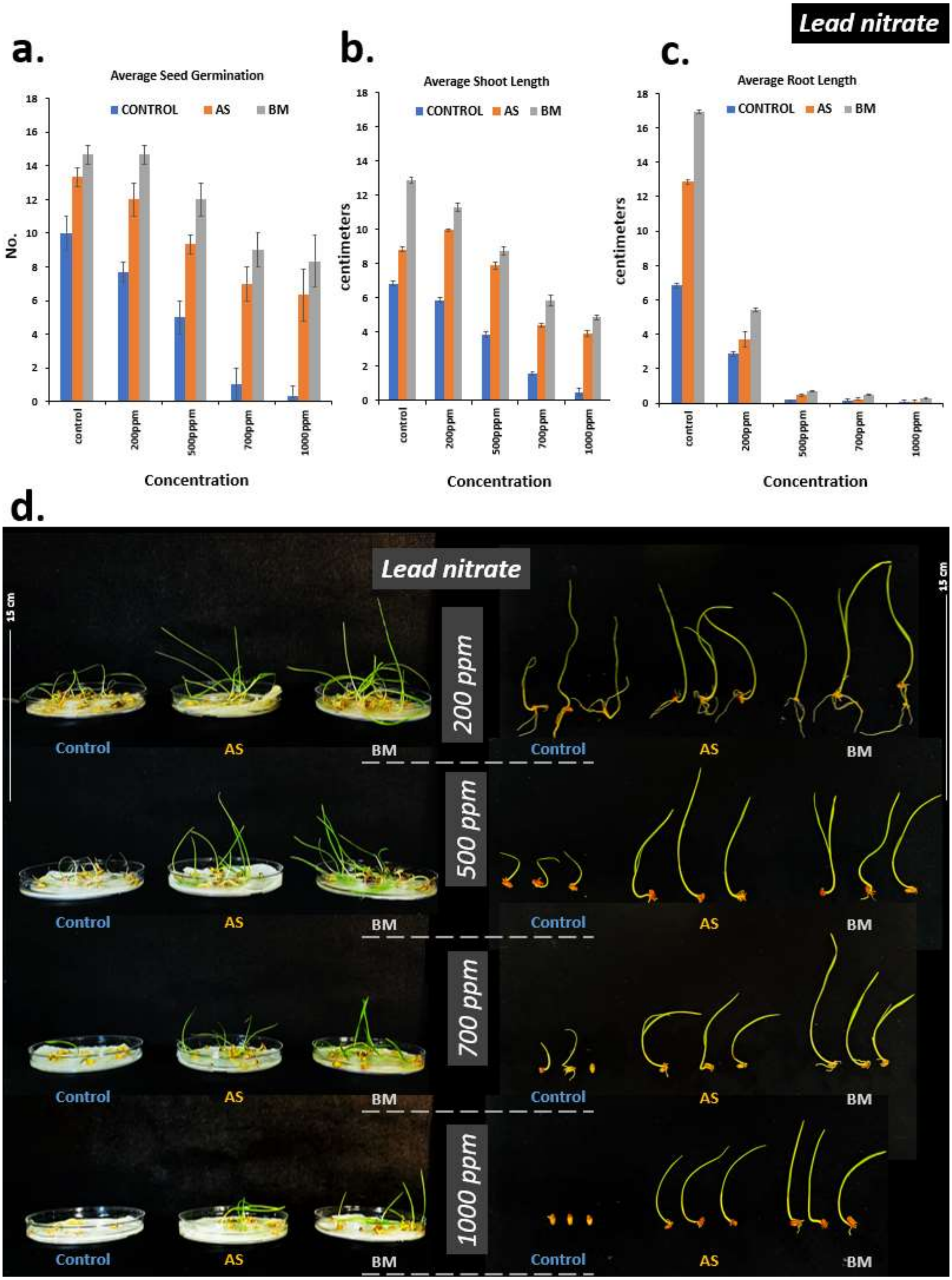

Fig. 3: Effect of seed bacterization treatments with EP1-AS and EP1-BM under various levels of the heavy metal lead nitrate. Here, the parameters shown are **a)** average germination from 20 wheat seeds per replicate per trial, **b**) shoot length development, and **c)** root length development. The results represent the averaged outcomes of three independent trials. In **d),** scale bars represent the lengths of seedlings under different variables.

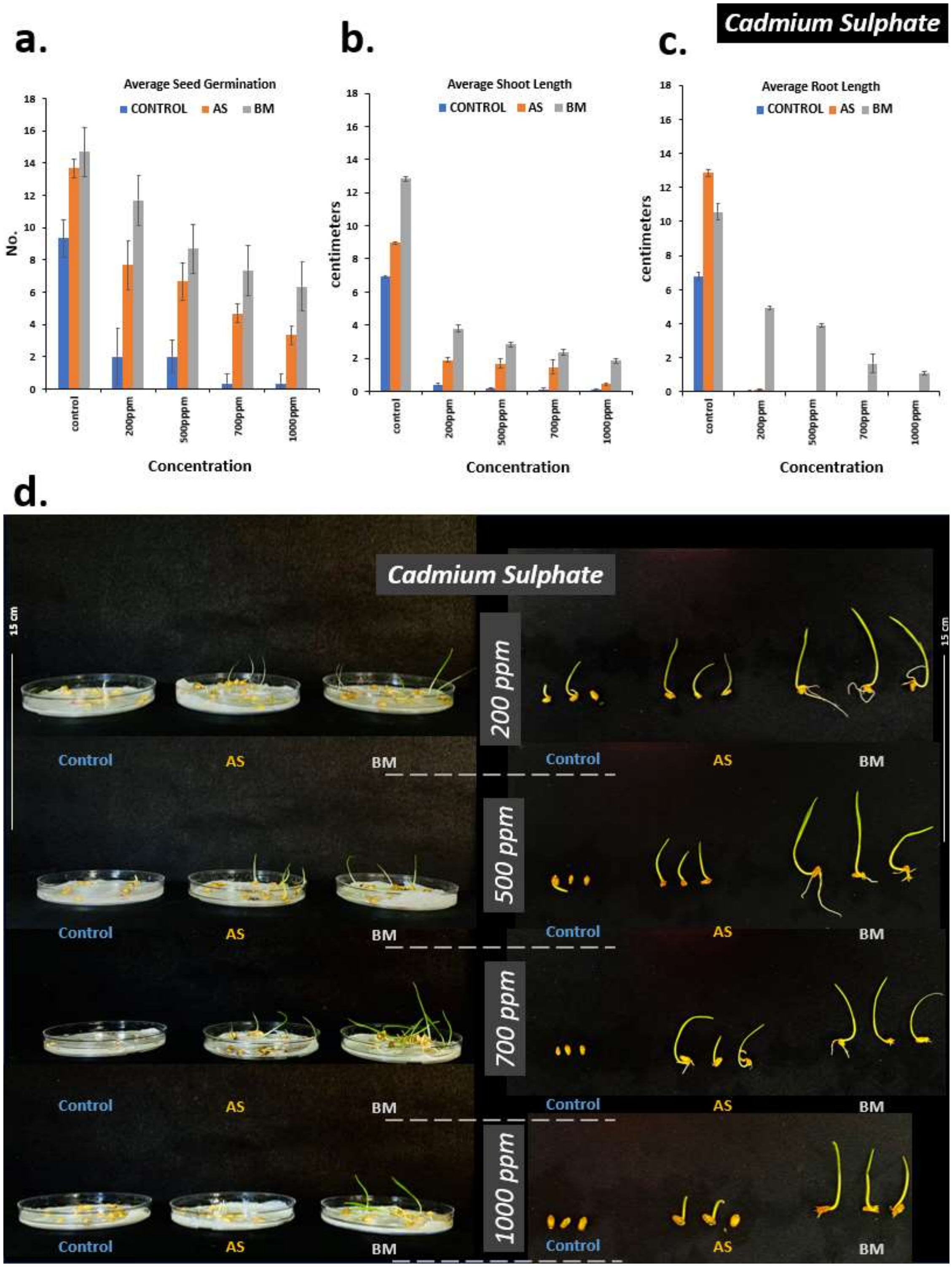

Fig. 4: Effect of seed bacterization treatments with EP1-AS and EP1-BM under various levels of the heavy metal Cadmium sulfate. Here, the parameters shown are **a**) average germination from 20 wheat seeds per replicate per trial, **b**) shoot length development, and **c**) root length development. The results represent the averaged outcomes of three independent trials. In **d**), scale bars represent the lengths of seedlings under different variables.

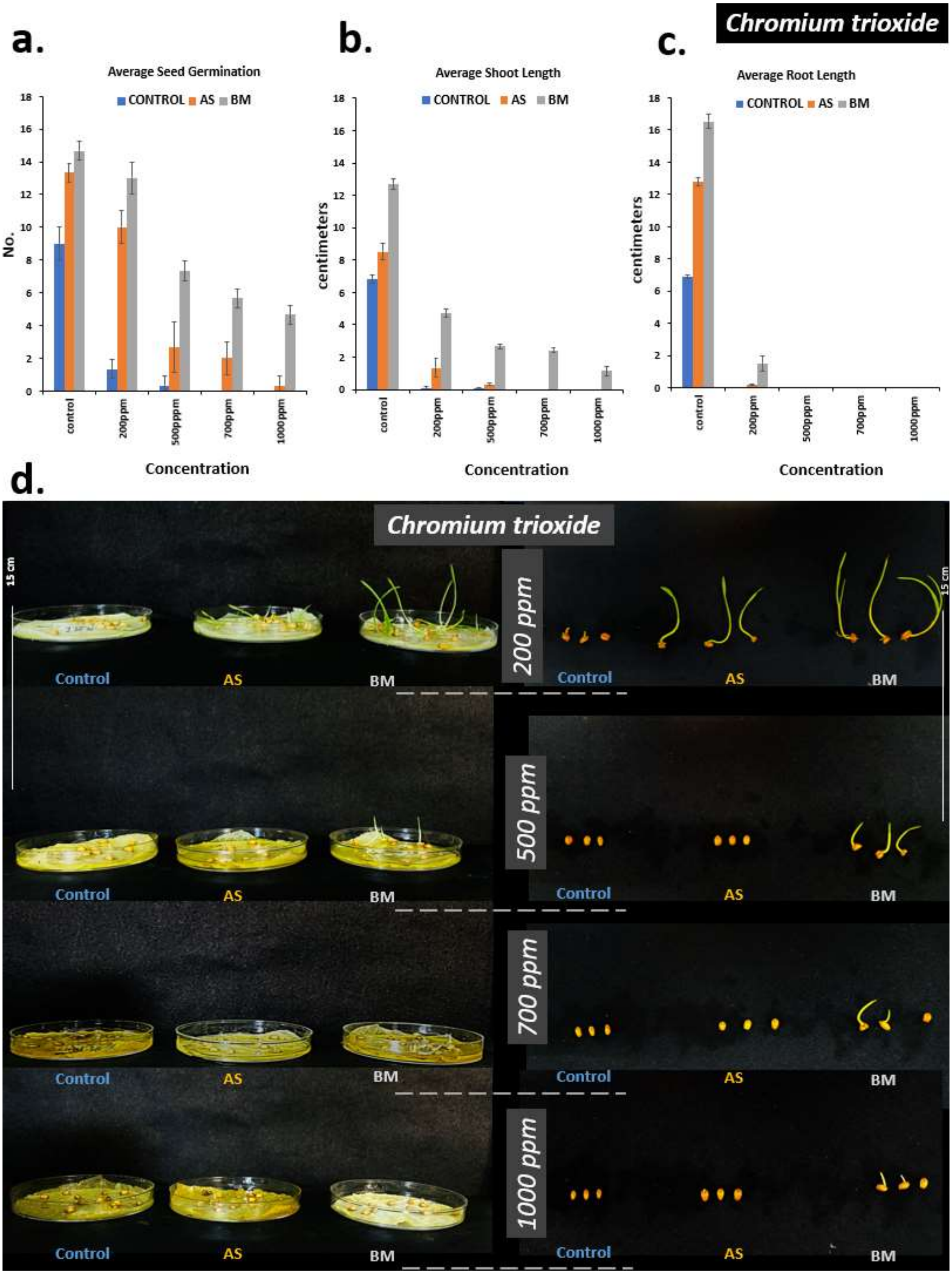

Fig. 5: Effect of seed bacterization treatments with EP1-AS and EP1-BM under various levels of the heavy metal chromium trioxide. Here, the parameters shown are **a**) average germination from 20 wheat seeds per replicate per trial, **b**) shoot length development, and **c**) root length development. The results represent the averaged outcomes of three independent trials. In **d**), scale bars represent the lengths of seedlings under different variables.

feature was only more prominent within the case with lead nitrate.

In contrast, in the case of sodium arsenite, the seedling shoot was observed to be more badly impacted, resulting in a very low length, which is easily differentiated under observations at 500 mM of either of these two heavy metals. This detrimentally also reflected well with seedlings' root development with comparatively much lower lengths at 200 mM itself for sodium arsenite than for lead nitrate (Fig. 2c and 3c). Beyond 200 mM, however, root growth was negligible and ceased completely in the case of either heavy metal. EP1-AS/EP1-BM treatments, however, could remarkably and significantly rescue the root growth in both cases of heavy metals, of which BM specifically and appreciably could hold much better root length even at 500 ppm and 700 ppm of sodium arsenite (Fig 2c) but for lead nitrate could only do better at 200 mM concentration (Fig 3c). The visual observation representations of the seedlings equated with the above results (Fig. 2d and 3d).

Effects with cadmium sulfate and chromium trioxide were more decrementing for the wheat seedling development (Fig. 4 and 5). In brief, control EP1-AS/EP1-BM untreated seed lots showed abrupt ceasing of germination at 200 ppm of either heavy metal such that only some 10 to 20% germination could be seen, especially at 500 ppm for chromium trioxide showed negligible seedling emergence compared to cadmium which still showed the emergence of the percentage as also in 200 ppm (Fig. 4a and 5a). Hence, comparatively, chromium trioxide seemed more problematic to seed germination success than cadmium sulfate. EP1-AS and EP1-BM treatments, however, seemed to enhance the germination profiles of seeds under treatments, but still, they can't be called, leading to productive germination. This is because shoot and root developments were badly impacted at any of the tested levels of heavy metals (Fig. 4b, 4c and 5b, 5c). Seedling shoot and root developments can also be seen and documented (Fig. 4d and 5d). EP1-BM as can be seen, maintained a higher rescuing ability for the shoot and root development in all levels of both the heavy metal.

Excess ions, such as sodium and chloride, can accumulate in plant tissues, disrupting crucial physiological processes (Malik et al. 2020). High salt concentrations compete with essential nutrients like potassium, calcium, and magnesium for plant roots. Similarly, heavy metals, even at low concentrations, can be toxic to plants and disrupt cellular processes, leading to oxidative stress (Asati et al. 2016). For instance, metals like cadmium inhibit photosynthesis, which is crucial for plant growth. The severity of these effects depends on factors like the type and concentration of salts or heavy metals, plant species, soil type, and environmental conditions. Plant species exhibit varying tolerance to salt and heavy metal stress, with some better adapted to such conditions.

The coexistence of two environmental pollutants, salt and heavy metals, significantly impacts plant development and growth. In wheat seedlings, salt stress at concentrations of 100 mM can decrease shoot length due to physiological changes like osmosis, ion imbalance, and oxidative stress, hindering cell division and elongation. Heavy metal pollution also detrimentally affects wheat seedling growth by impeding nutrient uptake, protein transport, and essential molecule synthesis, resulting in various growth defects (Bharti & Sharma 2022). The damage caused by salt and heavy metals depends on factors such as concentration, exposure duration, and genotype.

Salt stress disrupts cell osmotic equilibrium, causing cell shrinkage and death. Heavy metal pollution reduces root length by impairing water and nutrient uptake, damaging cell membranes, and generating free radicals that damage DNA and cellular proteins. The extent of root length reduction depends on pollutant concentration, exposure duration, and plant genetics. Understanding these complex interactions is crucial for developing strategies to mitigate the adverse impacts of salt and heavy metal stress on plant growth and overall health.

Within the domain of stress biology, ground-dwelling spurges have emerged as resilient species, displaying remarkable adaptability to challenging environmental conditions such as arid landscapes, saline soils, and polluted sites. From a biotechnological standpoint, these plants have become central subjects in translational research, particularly for applications in phytoremediation, bioenergy production, and phytomedicines. Our research is dedicated to uncovering the potential of endophytic microbial communities associated with robust succulent plants, with a specific emphasis on ground-dwelling spurges within the Euphorbiaceae family. This exploration led to the isolation of two distinct Lelliotia amnigena isolates, EP1-AS and EP1-BM, from internodal explants of the sandmat spurge, Euphorbia prostrata. Despite initial suspicions of contamination, these isolates demonstrated noteworthy plant-growth-promoting (PGP) properties, resulting in enhanced growth and productivity in wheat and tomato crops under ex vitro conditions. Notably, the EP1-BM isolate exhibited more pronounced PGP effects, further heightened through seed-priming treatments, showcasing improved in vitro plant regeneration efficiency in their latex-bearing spurge host. This enhancement extended to key physiological parameters, including chlorophylls, carotenoids, phenols, and flavonoids, signifying a holistic improvement in plant stress resilience and physiological vigor.

The promising results observed in ex vitro trials prompted an extended assessment through field testing, affirming the ability of EP1-AS and EP1-BM isolates to enhance crop productivity in wheat and tomato. While *L. amnigena* strains have been associated with pathogenesis in specific contexts, their general limited virulence factors suggest potential safety in practical applications. As we contemplate translating our PGPB findings into practical use, a cautious approach to pathogenesis and toxicity profiling is essential. Our innovative strategy involving cell-free spent supernatants of EP1-AS and EP1-BM isolates offers a novel avenue for developing biofertilizer formulations while addressing safety concerns. Importantly, our investigation has sparked intriguing inquiries regarding the colonization, dissemination, and endophytic potential of *Lelliotia* spp. in cultivated plants. The intricate interplay between these bacteria and their hosts, combined with the exploration of translational opportunities, emphasizes the need for rigorous and comprehensive research.

In our upcoming endeavors, we are expanding on this foundation, delving into the relationship between these isolates and their potential to enhance salt tolerance and heavy metal tolerance in crops, particularly focusing on wheat. By continuing to unravel the bioprospects from resilient plants and their associated microbial communities, we aim to pioneer sustainable agriculture and tap into the untapped potential of these often underestimated and robust plant species. These translational research endeavors are especially critical considering the global zero-hunger target by 2030 amidst the looming challenges of climate change and the broader Sustainable Development Goals (SDGs) outlined by the United Nations Organization (UNO).

CONCLUSION

In conclusion, this research article delves into the evaluation of Plant Growth-Promoting Bacteria (PGPB) strains EP1-AS and EP1-BM, isolated from the halophyte Euphorbia Prostrata, for their potential to enhance plant growth and provide abiotic stress resilience. The study addresses the pressing need for sustainable agricultural practices in the face of challenges like soil salinization and heavy metal contamination. The investigation involves a comprehensive analysis of the heavy metal and salt tolerance of the PGPB strains, shedding light on their potential applications in promoting plant growth under adverse environmental conditions. The research further explores the impact of these PGPB strains on wheat plants subjected to varying concentrations of heavy metals and salts. Results indicate that both PGPB strains, especially EP1-BM, exhibit significant tolerance to heavy metals and salt stress. EP1-BM demonstrates remarkable resilience even under high concentrations of these stressors. The study extends its findings to in vitro testing on wheat plants, revealing the positive influence of PGPB strains on germination, shoot length, and root length in the presence of salt and heavy metals.

The research underscores the significance of understanding plant-microbe interactions, particularly in the context of promoting sustainable agriculture in challenging environments. The identified resilience of PGPB strains, especially EP1-BM, suggests their potential application as bio-remediators and plant growth promoters in soils affected by salinity and heavy metal stress.

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DATA AVAILABILITY

The homologs of the 16S rRNA gene sequences corresponding to the EP1-AS and EP1-BM isolates have been securely deposited in the GenBank database hosted by the National Center for Biotechnology Information (NCBI), with accession numbers OR342320 and OR342321, respectively. In adherence to transparency and scientific openness, the corresponding authors commit to providing any raw data that underpins the findings of this article. Interested parties are invited to formally request access to the data, and such requests will be accommodated promptly and without undue reservation. This commitment ensures the accessibility and reproducibility of the study’s results, fostering a collaborative and accountable approach to scientific research.

REFERENCES

Amtaghri, S., Akdad, M., Slaoui, M. and Eddouks, M. 2022. Traditional uses, pharmacological, and phytochemical studies of Euphorbia: A review. Curr. Top. Med. Chem., 22: 1553-1570.

Asati, A. 2016. Effect of heavy metals on plants: An overview. Int. J. Apppl. Innov. Eng. Manag., 5(3): 56-66.

Bharti, R. and Sharma, R. 2022. Effect of heavy metals: An overview. Mater. Today Proceed., 51: 880-885.

Chan, A.P., Crabtree, J., Zhao, Q., Lorenzi, H., Orvis, J., Puiu, D., Melake-Berhan, A., Jones, K.M., Redman, J., Chen, G., Cahoon, E.B., Gedil, M., Stanke, M., Haas, B.J., Wortman, J.R., Fraser-Liggett, C.M., Ravel,

J. and Rabinowicz, P.D. 2010. Draft genome sequence of the oilseed species *Ricinus communis*. Nature Biotechnol., 28: 951-956

Chaudhary, P., Singh, D., Swapnil, P., Meena, M. and Janmeda, P. 2023. *Euphorbia neriifolia* (Indian Spurge Tree.: A plant of multiple biological and pharmacological activities. Sustainability, 15: 1225

Dubyna, D.V., Ennan, A.A.A., Dziuba, T.P., Vakarenko, L.P., Shykhaleyeva, G.M. and Kiryushkina, H.M. 2022. Anthropogenic transformations of vegetation in the Kuyalnik estuary valley (Ukraine, Odesa District). Diversity, 14: 1115.

Linfang, H., Shilin, C. and Meihua, Y. 2012. *Euphorbia hirta* (Feiyangcao): A review on its ethnopharmacology, phytochemistry and pharmacology. J. Med. Plants Res., 6: 5176-5185

Jeevanandam, J. 2021. Biosynthesized metal nanoparticles in bioremediation. Bioremediation, 5: 126-161.

Kaur, J., Dhar, S.K., Chauhan, A., Yadav, S., Mudgal, G., Lyudmila, A., Atuchin, V. and Abdi, G. 2023a. GC-MS validated phytochemical up-leveling with in vitro-raised *Sansevieria trifasciata*. Current Plant Biology, 35-36: 100308

Kaur, J. and Mudgal, G. 2020a. An efficient protocol for micropropagation of small-flowered bugleweed. Plant Cell Biology and Molecular Biology, 5: 16-22.

Kaur, J. and Mudgal, G. 2020b. Phytochemistry and pharmacological assets of Ziziphus Juazeiro-Mart: A review. Plant Cell Biol. Mol. Biol., 16: 112-126.

Kaur, J. and Mudgal, G. 2021. An efficient and quick protocol for in vitro multiplication of snake plant, *Sansevieria trifasciata* var. laurenti. Plant Cell, Tissue and Organ Culture, 147: 405-411.

Kaur, J., Mudgal, G., Chand, K., Singh, G.B., Perveen, K., Bukhari, N.A., Debnath, S., Mohan, T.C., Charukesi, R. and Singh, G. 2022. An exopolysaccharide-producing novel *Agrobacterium pusense* strain JAS1 isolated from a snake plant enhances plant growth and soil water retention. Sci. Rep., 12: 21330

Kaur, J., Mudgal, G., Negi, A., Tamang, J., Singh, S., Singh, G.B., Bose K., J.C., Debnath, S., Wadaan, M.A., Farooq Khan, M., Ruokolainen, J. and Kesari, K.K. 2023b. Reactive Black-5, Congo red, and methyl orange: Chemical degradation of azo-dyes by Agrobacterium. Water, 15: 1664

Kaur, J. 2020. Genetic Transformation of *Olea europaea* (Olive). for folate biofortification. Plant Cell Biol. Mol. Biol., 16: 72-82.

Khaksar, G., Siswanto, D., Treesubsuntorn, C. and Thiravetyan, P. 2016. Euphorbia milii-endophytic bacteria interactions affect the hormonal levels of the native host differently under various airborne pollutants. Mol. Plant Micro. Interact., 29: 663-673

Malik, A., Butt, T.A., Naqvi, S.T.A., Yousaf, S., Qureshi, M.K., Zafar, M.I., Farooq, G., Nawaz, I. and Iqbal, M. 2020. Lead tolerant endophyte *Trametes hirsuta* improved the growth and lead accumulation in the vegetative parts of Triticum aestivum L. Heliyon, 6: e04188.

Mingo-Castel, A. 1984. Hormonal effects on phyllotaxis of Euphorbia lathyris L. Botan. Mag., 97: 171-178.

Mudgal, G., Kaur, J., Chand, K., Parashar, M., Dhar, S.K., Singh, G.B. and Gururani, M.A. 2022. Mitigating the mistletoe menace: Biotechnological and smart management approaches. Biology, 11: 1664

Mudgal, G. 2021. The Antioxidant Arsenal against COVID-19. Springer International Publishing, Cham, Germany, pp. 327-357.

Mudgal, G. and Mudgal, B. 2011. Evidence for the unusual choice of host and haustoria by Dendrophthoe falcata (L.f. Ettingsh), a leafy mistletoe. Arch. Phytopathol. Plant Protect., 44: 186-190.

Mudgal, G., Mudgal, B., Gururani, M.A. and Jelli, V. 2011. Pseudaulacaspis cockerelli (Cooley. hyperparasitizing Dendrophthoe falcata (L.f.) Ettingsh. Arch. Phytopathol. Plant Protect., 44: 282-286.

Parashar, M., Dhar, S.K., Kaur, J., Chauhan, A., Tamang, J., Singh, G.B., Lyudmila, A., Perveen, K., Khan, F., Bukhari, N.A., Mudgal, G. and Gururani, M.A. 2023. chapters 2 Novel plant-growth-promoting *Lelliottia amnigena* isolates from Euphorbia prostrata Aiton enhance the overall productivity of wheat and tomato. Plants, 12: 3081

Parashar, M., Mudgal, G. and Jaspreet, K. 2020a. Phytochemical profiling, antimicrobial and antioxidant activities on leaves extract of *Murraya koenigii*. Plant Cell Biol. Mol. Biol., 303-313.

Parashar, M., Mudgal, G. and Sharma, S. 2020b. Bioremediation using green approaches with *Euphorbia prostrata*: A review. Plant Cell Biol. Mol. Biol., 21: 127-135.

Pascal, O.A. 2017. A review of the ethnomedical uses, phytochemistry, and pharmacology of the Euphorbia genus. J. Pharm. Innov., 6: 1, 34.

Ramamoorthy, S., Mudgal, G., Rajesh, D., Nawaz Khan, F., Vijayakumar, V. and Rajasekaran, C. 2009. Characterization of novel pH indicator of natural dye Oldenlandia umbellata L. Natural Prod. Res., 23: 1210-1217.

Saeed, T., Khan, T.A., Ahmad, A., Yusuf, M., Kappachery, S., Fariduddin, Q., Mudgal, G. and Gururani, M.A. 2023. Exploring the effects of selenium and brassinosteroids on photosynthesis and protein expression patterns in tomato plants under low temperatures. Plants, 12: 3351.

Salatino, A., Salatino, M.L.F. and Negri, G. 2007. Traditional uses, chemistry, and pharmacology of *Croton* species (Euphorbiaceae). J. Brazil. Chem. Soc., 18: 11-33.

Sharma, A. 2021. Toxicity with Waste-Generated Ionizing Radiations: Blunders Behind the Scenes. Free Radical Biology and Environmental Toxicity. Springer International Publishing, Cham, Germany, pp. 305-325.

Singh, G.B. 2021. Molecular Communications Between Plants and Microbes. Plant-Microbial Interactions and Smart Agricultural Biotechnology. CRC Press, Boca Raton, USA, pp. 147-184.

Siva, R., Rajasekaran, C. and Mudgal, G. 2009. Induction of somatic embryogenesis and organogenesis in *Oldenlandia umbellata* L., a dye-yielding medicinal plant Plant Cell Biol. Mol. Biol., 98: 205-211.

Sivakumaran, S. and Hall, M.A.1978. Effects of age and water stress on endogenous levels of plant growth regulators in *Euphorbia lathyrus* L. J. Exper. Bot., 29: 195-205.

Srivastava, R. and Soni, N. 2019. An updated review on the phytopharmacological profile of *Euphorbia tithymaloides* (L). Poit. Pharma Innov. J., 8: 109-115.

Unde, S. and Kumar, S.R. 2022. Phytoremediation potentials of some ornamental plants: A review. Adv. Innov. Res., 85: 14-20.

Vinayak, A. 2020. Waste to energy: Sustainable bioremediation approach for renewable energy. Plant Cell Biol. Mol. Biol., 11: 292-302.

Vinayak, A. 2021. Environment Persistent Free Radicals: Long-Lived Particles. Free Radical Biology and Environmental Toxicity. Springer International Publishing, Cham, Germany, pp. 1-19.

Washington, J. 1980. Dilution susceptibility test: Agar and macro-broth dilution procedures. Am.. Soc. for Microbiol., 11: 2461.

Webster, G.L. 1967. The genera of Euphorbiaceae in the southeastern United States [concl.]. J. Arnold Arboretum, 48: 363-430.