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Pseudochrobactrum asaccharolyticum mitigates arsenic induced oxidative stress of maize plant by enhancing water status and antioxidant defense system

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Abstract

Background Oxidative stress mediated by reactive oxygen species (ROS) is a common denominator in arsenic toxicity. Arsenic stress in soil affects the water absorption, decrease stomatal conductance, reduction in osmotic, and leaf water potential, which restrict water uptake and osmotic stress in plants. Arsenic-induced osmotic stress triggers the overproduction of ROS, which causes a number of germination, physiological, biochemical, and antioxidant alterations. Antioxidants with potential to reduce ROS levels ameliorate the arsenic-induced lesions. Plant growth promoting rhizobacteria (PGPR) increase the total soluble sugars and proline, which scavenging OH radicals thereby prevent the oxidative damages cause by ROS. The main objective of this study was to evaluate the potential role of Arsenic resistant PGPR in growth of maize by mitigating arsenic stress.

Methodology Arsenic tolerant PGPR strain MD3 (*Pseudochrobactrum asaccharolyticum)* was used to dismiss the 'As' induced oxidative stress in maize grown at concentrations of 50 and 100 mg/kg. Previously isolated arsenic tolerant bacterial strain MD3 "*Pseudochrobactrum asaccharolyticum* was used for this experiment. Further, growth promoting potential of MD3 was done by germination and physio-biochemical analysis of maize seeds. Experimental units were arranged in Completely Randomized Design (CRD). A total of 6 sets of treatments viz., control, arsenic treated (50 & 100 mg/kg), bacterial inoculated (MD3), and arsenic stress plus bacterial inoculated with three replicates were used for Petri plates and pot experiments. After treating with this MD3 strain, seeds of corn were grown in pots filled with or without 50 mg/kg and 100 mg/kg sodium arsenate.

Results The plants under arsenic stress (100 mg/kg) decreased the osmotic potential (0.8 MPa) as compared to control indicated the osmotic stress, which caused the reduction in growth, physiological parameters, proline accumulation, alteration in antioxidant enzymes (Superoxide dismutase-SOD, catalase-CAT, peroxidase-POD), increased MDA content, and H_2O_2 in maize plants. As-tolerant *Pseudochrobactrum asaccharolyticum* improved the plant growth by reducing the oxidation stress and antioxidant enzymes by proline accumulation. PCA analysis revealed that all six treatments scattered differently across the PC1 and PC2, having 85.51% and 9.72% data variance, respectively. This indicating the efficiency of As-tolerant strains. The heatmap supported the As-tolerant strains were positively correlated with growth parameters and physiological activities of the maize plants.

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Conclusion This study concluded that *Pseudochrobactrum asaccharolyticum* reduced the 'As' toxicity in maize plant through the augmentation of the antioxidant defense system. Thus, MD3 (*Pseudochrobactrum asaccharolyticum*) strain can be considered as bio-fertilizer.

Keywords Arsenic, Membrane Stability Index, Plant Growth Promoting Rhizobacteria

Introduction

Arsenic (As) is a category I carcinogen and a prevalent environmental toxin found in both soil and water. 'As' is a not an essential element for plants and primarily found as arsenite (As III) and arsenate (As V). This is a powerful and highly dangerous carcinogen for multicellular organisms [1]. Arsenic pollution of ground water is a worldwide problem that causes a variety of health issues [2]. Both anthropogenic and natural activities contribute to the accumulation of 'As' in the environment [3]. High level of 'As' in plants are widely known to cause toxicities [4]. Arsenic pollution is widespread throughout the world, particularly in the USA, Taiwan, China, Bangladesh, Brazil, France, India, China, and Malaysia [5]. Arsenic exposure has become a major public health concern in Pakistan. In Punjab, more than 20% of the population faces arsenic levels in drinking water above 10 ppb, whereas only around 3% encounter levels exceeding 50 ppb [6]. Continuous exposure of high and low level can lead to a variety of life-threatening conditions (neurologic, pulmonary, and cardiovascular issues along with other types of malignancies) [7]. International Agency for Research on Cancer (IARC) has classified arsenic and arsenic compounds as carcinogenic in food and drinking water.

Arsenic poisoning is characterized by oxidative stress mediated by reactive oxygen species (ROS) [8]. Arsenic, in particular, has an important function in plant water relations and water availability in soils, altering water intake and initiating a cascade of stress situations [9]. The osmotic potential of arsenic-contaminated soil decreases relative to the cell sap inside the root system. Under these conditions, the soil solution significantly impede the water intake in plants and create osmotic stress, which causes stomata to close, reduce CO_2 availability, photosynthesis and increase the ROS production in chloroplasts [10]. Osmotic stress also elevate the levels of MDA and H_2O_2, which are considered stress indicators. The 'As'-ROS-cell signaling axis influences the germination, biochemical and physiological processes, changes in antioxidant enzymes, lipid peroxidation, and physiological processes e.g., ATP depletion, excess ROS production, mitochondrial respiratory chain damage, oxidative phosphorylation decoupling and mitochondrial death [11].

Zea mays L., known as maize or corn belongs to C4 plants of Poaceae (Gramineae) family [12]. It is a third-most important cereal crop after rice and wheat [13]. *Zea mays* plays an important role in world's agricultural food system like food and nutrition security [14]. Globally, in 2021, 197 million hectares of maize were grown for grain production [15]. Polluted environmental conditions and heavy metal-contaminated soils are creating major difficulties in maize production [16]. Arsenic is a dangerous metalloid, reducing maize growth and production at alarming level [17]. Pakistan ranks fourth in maize cultivation but a definite drop in the yield due to heavy metals and metalloid pollution is occurring. Aside from polluted drinking water, the consumption of these 'As'infected cereals (*Oryza sativa* L., *Triticum aestivum* L., *Zea mays* L.) and pulses are causing major health issues in human beings. There is an urgent need to address arsenic toxicity and threats by green environment friendly and cost-effective techniques.

Tolerance of plants to ROS-induced oxidative stress is important for 'As' tolerance. Thus, there is a need to address the hazards of arsenic in crop production by viable economical methods. Several conventional remediation technologies e.g., chemical precipitation, coagulation wetland system, adsorption and electrochemical treatment have been investigated in recent decades [18], but these are traditional and not cost effective physicochemical approaches. Moreover, these techniques are non-effective and suitable for large-scale applications. In these circumstances, microbial remediation is a biological method for removal of harmful metals by fungi, algae and bacteria [19]. PGPR is a type of soil bacteria that colonizes in plant roots and increases the plant growth and yield. Plants with a stronger antioxidant defense mechanism are more resistant to 'As' poisoning. PGPR inoculation increase the proline (amino acid) content, which assist the plants in water status [20]. Proline is a powerful non-enzymatic antioxidant [21], singlet oxygen quencher and OH radical scavenger; when accumulated in plant tissue plays a significant role in reducing the oxidative damage. PGPR significantly increase the activity of antioxidant enzymes (ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase, catalase (CAT), monodehydroascorbate reductase, peroxidase, and superoxide dismutase) that scavenge ROS [22]. By redox and dismutation of O_2 to H_2O_2, these enzymes

mitigate the deleterious effects buildup by the ROS [23].

Arsenic-tolerant PGPR can alleviate the arsenic problems in crops and improve the plant health. In soil, PGPR biotransform toxic 'As' species into less detrimental ones, prevent the entrance of new harmful 'As' species into grains, and mitigate the negative effects by sequestering the arsenic. 'As' tolerant PGPR are not only helpful in agriculture, but also enhance the plant development and safe food via cost-effective manners [24]. Similarly, proline producing 'As' tolerant PGPR reduces the oxidative stress, protect the photosynthetic apparatus, and enhances the plant's antioxidant system by alleviating the heavy metal stress [25].

In the present scenario, starvation of food force the scientists to explore neglected areas ('As' contaminated lands) for crop production. Extreme 'As' toxicity now becomes a global concern due to health risks. These problems are forcing the global concern towards environmental friendlier approaches. One of the acceptable alternative is to search 'As' tolerant PGPR, which reduce arsenic stress in plants. Such PGPR are widely known for their ability to promote plant development. The target of this current study was to investigate the effects of arsenic tolerant PGPR strain (MD3) to reduce the ROS in maize plant via modulation of antioxidant enzymes indirectly improve the growth and physiological attributes under different levels of arsenic contaminated soil.

Materials and methods

Two sets of petri plate and pot experiments were conducted to alleviate the arsenic stress by PGPR in *Zea mays* L. Maize seeds (certified variety DK-6148) were collected from Punjab Seed Corporation Lahore taken 85–100 days in physical maturity with excellent MLND tolerance. Petri plate experiment was conducted during corn growing season in the Plant physiology laboratory, LCWU, Lahore. There was a total 6 sets of treatments with three replicates. A pot experiment accomplished to evaluate the effects of arsenic and bacterial inoculation on different physiological and biochemical parameters of maize. Sampling was done at germination and reproductive stages. For each test, leaves of plants were taken from each treatment and after weighing stored at -20 °C in freezer for further analysis. The equipment used in this study were purchased from GM Scientific Linkers.

Inoculum preparation, seed sterilization, and seed-coating

Potential strain of PGPR, *i.e., Pseudochrobactrum asaccharolyticum* (MD3= isolated from the soil collected from Muridke, Pakistan) was selected for in vitro studies. The strain was identified by PCR-amplified 16S rRNA gene products (1500 bp) of the chosen isolate which was compared by using NCBI database BLAST-N program and the sequence was submitted to NCBI database. Neighbor-joining technique was used to construct the phylogenetic tree via MEGA X software.

For the collection of pellet, culture was taken after 48 h, centrifuged at 10,000g for ten min. The supernatant was discarded and pellet was then resuspended in distilled water. The bacterial solution was diluted to 0.1 and OD was taken at 600 nm for homogenous bacterial population (10^8 CFU mL^{-1}) (Table 1).

Maize seeds were disinfected by serial washing for 1 min with 0.1% $HgCl_2$ and 70% ethyl alcohol for 5 min. After disinfection, all seeds were rinsed three times with autoclaved distilled water. For sticking bacteria onto the seed surface, half mL of 10% arabia gum was put on the seeds. After a while, bacterial suspension (1 mL) was added in 15 g of maize seeds and left for 2–3 h. In control group, seeds were dipped in distilled water.

Germination test

Petri plate experiments were conducted in properly washed and autoclaved petri plate. Plates were dried in oven at 170 °C for 60 min. After sterilization of petri plates, 20 seeds were placed on filter paper of each petri plate with respective solutions and kept at room temperature (20 ± 2 °C). Petri plates are were organized in Completely Randomized Design (CRD) and germination of maize seeds in each treatment was recorded on daily basis up to six days and then germination parameters were assessed.

Growth parameters of plants

For checking reduction of arsenic stress in maize by PGPR strain, leaf samples from Petri plates were taken to note the following growth parameters:

1. Germination Percentage

Germination was measured by counting No. of seeds that sprouted in every petri dish. Germination Percentage was calculated by following equation [26].

$$\text{Germination percentage} = \frac{\text{No. of seed germinated during the time interval}}{\textit{Total No. of seeds sown}} \times 100 \quad (1)$$

2. Germination rate

The following equation (Eq. 2) was used to compute the germination rate [27].

$$\text{Germination Rate} = \sum g/t \quad (2)$$

whereas,

g indicates percentage of seed germinated every day and t refers to total germination period.

3. Fresh weight & dry weight of seedlings

For the determination of dry and fresh weight (g), three seedlings from each petri plate were selected randomly after six days of seeds sowing period. Fresh weights (g) noted by electrical balance and weights of oven dried (70 °C for 72 h) seedlings were estimated till weight becomes constant.

4. Root and shoot length of seedlings

Prior to seed sowing, after six days, three plants were selected randomly from each Petri plates. Shoot and Root length (cm) were noted by ruler.

5. Seedling Vigor Index (SVI)

Seedling length was firstly taken in cm and then multiply seedling length with germination percentage for the assessment of SVI by following equation [28].

$$\text{Seedling Vigor Index} = \text{seedling length x germination percentage} \quad (3)$$

6. Tolerance indices

From each Petri plate, randomly three seedlings were selected and noted for their root length [29] by following equation (Eq. 4) used to determine the tolerance index of leaves.

$$\text{Tolerance index} = \text{Mean root length in the treatment}/\text{Mean root length in the control} \times 100 \quad (4)$$

Table 1 Optical density of bacterial isolate

Dilutions	OD (Optical Density)
MD3 (1)	0.1174
MD3 (2)	0.85211
MD3 (3)	1.1192
MD3 (4)	0.7139

Colony Forming Unit: CFU/mL = colonies × Final Dilution Factor

$125 \times (8 \times 10^4) = 1 \times 10^8$ CFU/mL ($OD_{600} = 0.1$)----MD3(1)

Pot experimental design

Pot experiment was performed at Botanical Garden, LCWU, Lahore in order to determine how Rhizobacteria enhanced the maize development in arsenic contaminated soil. The sandy clay loamy soil was collected from local supplier. The physico-chemical properties like Electrical Conductivity, pH, organic matter, micro and macro-nutrient composition, were calculated after one month (Table 2).

To get rid of all bacterial and fungal spores, the soil was first air dried in the lab, then crushed, sieved and sterilized. Sterilized soil (10 kg) filled in each pot lined with polythene sheet and seeds were sown (5 surfaces-sterilized seeds per pot). The application of arsenic in sodium arsenate form at 50 mg/kg and 100 mg/kg was done. World Health Organizations (WHO) indicated arsenic level in drinking water is 10 ug/L, while in all developing countries concern with polluted groundwater with recent WHO-guideline indicated 50 ug/L. value. Arsenic concentrations (50 mg/kg and 100 mg/kg) were slowly and progressively mixed in ground dry soil of respective treatments and the mixture were continued to expand to cover the soil. Finally, the spiked soil mixture was homogenized through diagonal flipping soil on the plastic sheet for 3 to 5 times. The most common species of 'As' in soils is Arsenate (Na_3AsO_4) a less toxic form than sodium arsenite. Total 6 treatments viz., control, arsenic treated 50 mg/kg and 100 mg/kg As), PGPR inoculated *Pseudochrobactrum asaccharolyticum*), arsenic plus PGPR inoculation (50 mg/kg As + Isolate and 100 mg/kg As + Isolate) with three replicates were used for pot experiment. In Pot experiment, sampling was done at reproductive stage, and roots were washed under running tap water for the removal of soil particles. After this, the roots were bagged for further testing.

Physio-biochemical studies of plants

After 9 weeks of seed germination, leaf samples were collected from each treatment for determination of various physio-biochemical attributes.

1. Relative Water Content (RWC)

Weighing balance was used to record the fresh leaf weights from each replicate of six treatments. The leaves were submerged in distilled water for 24 h in petri plates. The fully turgid leaves were weighed to determine the saturated mass and then dried in oven for 72 h at 70 °C [30]. The following equation (Eq. 5) was used to determine RWC of the leaves.

Table 2 Chemical analysis of soil before and after 'As' spiking in Pot Experiment

Characteristics	Value before spiking	Value after spiking
pH	7.6	5.2
Organic matter	0.5499%	0.2844%
Electrical Conductivity	250 S/m	220 S/m
Available phosphorus	6.5 mg/kg	3.4 mg/kg
Total Nitrogen	0.05%	0.02%
Extractable potassium	120.5 mg/kg	95.8 mg/kg

$$\text{RWC} = [(\text{fresh mass} - \text{dry mass})/(\text{saturated mass} - \text{dry mass})] \times 100 \quad (5)$$

2. Osmotic potential

The osmotic potential was determined in vapor pressure depression VAPRO 5520 Wescor osmometer for cell sap of maize leaves [31]. Before freezing, leaves were cleaned and placed in a 2 mL syringe. After 24 h, the syringes were taken from the refrigerator and pressed to extract 50 µL cell sap from the thawed leaves. The primary osmometer measurement was in mosmol/kg, which converted into megapascles (MPa) units by using following formula (Eq. 6):

$$\text{Osmotic potential of cell's sap} = \text{osmolality (mosmol)} \times 0.831 \times 10^{-5} \times \text{T (K)} \quad (6)$$

3. Membrane Stability Index (MSI)

Wash 100 mg of leaf discs twice from each treatment: once with tap water and once with double-distilled water. After washing, the leaf discs were placed in a water bath set at 40 °C for 30 min by using 10 mL of double-distilled water. During this time, electrical conductivity (C1) was measured with EC meter. The same samples were then heated to 100 °C in a boiling water bath for 10 min. After 10 min, its electrical conductivity (C2) was also measured. The membrane stability index (MSI) was calculated by using following equation (Eq. 7) below [32, 33].

$$\text{MSI} = \left(1 - \frac{C1}{C2}\right) \times 100 \quad (7)$$

4. Sugar content

The 0.5 g of leaf material from each treatment was weighed and crushed in a clean Pestle and mortar by adding 10 mL of purified water. This homogenized material was filtered and 0.1 mL of the filtrate from each treatment was mixed in 1 mL of phenol (5% v/v). This mixture was then incubated at room temperature for 1 h. After an hour, the test tubes were filled with 5 mL of conc. H_2SO_4. Each sample was placed in the cuvette of spectrophotometer and absorbance measured at 420 nm. The concentration of sugar was estimated by using a standard curve for a known concentration of glucose solution [34].

5. Proline content of leaves

Maize leaves were collected at reproductive stage, and the proline content of these leaves was then calculated [35].

Proline extraction protocol

From every treatment, 0.1 g of freeze-dried leaf material was mixed in 3.0% sulfosalicylic acid (4 mL) in a clean pestle and mortar. The suspension was then centrifuged for 5 min at 3000 rpm at room temperature. The 4 ml acetic ninhydrin reagent was added to the supernatant. To make 6 M orthophosphoric acid, 28.5 mL of orthophosphoric acid (85%) was added in 12 mL distilled water, and then glacial acetic acid (180 mL) was added in this mixture. The contents of the tubes were heated in a boiling water bath for an hour at 50 °C (overnight), with continuous stirring the reaction mixture. This chilled test tube content in a separating funnel with 4 mL of toluene was used for separation. The tuolene layer was added in cuvette of spectrophotometer and absorbance at 520 nm was measured. These unidentified proline in samples were calculated by proline standard curve.

6. Protein content of leaves

Maize leaves were collected at reproductive stage and the protein content was determined by BSA standard of bovine serum albumin [36].

Protein extraction protocol

For the protein content, leaf material from each treatment was crushed in Pestle and mortar with 1 mL of sodium phosphate buffer (pH 7.5). This mixture was centrifuged at 3000 rpm for 10 min. The supernatant

(0.1 mL) from each treatment was then collected and placed in test tubes. Fill each test tube with 0.9 mL distilled water to make the volume upto 1 mL and each test tube then filled with 1 mL of reagent C. Following that, the reaction mixture was removed from each test tube and shaken for 10 min. After that, the test tubes were filled with reagent D (0.1 mL) and allowed to rest at room temperature for 30 min and absorbance noted at 650 nm. The protein concentration in the samples was calculated by using BSA standard curve.

7. Estimation of lipid peroxidation

The production of TBARS (thiobarbituric acid reactive chemicals) for lipid peroxidation can be utilized to determine the degree of lipid peroxidation [37]. After fine crushing, 2 mL of 0.1% trichloroacetic acid mixed in 0.1 g of leaf samples and then centrifuged for 15 min. One mL supernatant, 4 mL of 0.5% thiobarbituric acid, and 1 mL of 20% TCA mixture was heated for 30 min in a water bath (95°C). The mixture was then placed in an ice bath to cool. Following ten minutes of centrifugation, the absorbance of the supernatant was measured at 440, 532 and 600 nm. The given formula was used to determine malondialdehyde equivalents given in nmol ml^{-1} [38]:

$$MDA = \frac{(A532 - A600) - \left\{(A440 - A600)\left(\frac{MA\ of\ sucrose\ at\ 532nm}{MA\ of\ sucrose\ at\ 440nm}\right)\right.}{157000} \times 106 \quad (8)$$

The MDA (molar absorbance) of 1–10 mM sucrose at 532 nm and 440 nm was determined to be 8.6 and 149, respectively.

8. Determination of antioxidant enzymes activities

Preparation of enzyme extract

For antioxidant enzyme assay, 0.5 g of maize leaves were crushed in pestle and mortar over an ice bath and then homogenized in 5 mL of phosphate buffer (50 mM). The supernatant was used to assess the enzyme activity after centrifuging the mixture at 13,000 rpm for 20 min at 4°C.

a Superoxide Dismutase (SOD) activity

The ability of superoxide dismutase (SOD) was used to prevent photochemical reduction of nitroblue tetrazolium (NBT) [39]. Each sample's reaction mixture (2 mL) comprised 0.2 mL methionine (13 mM), 0.1 mL NBT (0.075 mM), 0.2 mL triton X, 0.1 mL riboflavin (0.002 mM), and 0.1 mL of enzyme extract in 0.5 mL of 50 mM phosphate buffer (pH 7.8), 50 uL, 75 uM, 2 uM, 0.1 mM EDTA reaction mixture was irradiated for 15 min under UV light before the reaction stopped by removing the samples from the light source. In a control set, a blank of non-illuminated reaction mixture was employed. The absorbance of the samples were measured by using a dual beam spectrophotometer at 560 nm. One unit of superoxide dismutase activity was defined as the amount of enzyme that inhibits 50% absorbance compared to the control and expressed as units per 100 mg of fresh weight. The following formula was used to compute the superoxide dismutase activity (Eq. 10).

$$IU = \frac{absorbance}{50} \times 10 \quad (9)$$

IU is an abbreviation for international unit of enzyme activity.

$$SOD\ activity = \frac{IU}{mg\ of\ protein} \quad (10)$$

b Catalase (CAT) activity

The 3 mL of reaction mixture was taken, which comprised 2.6 mL of 50 mM phosphate buffer (pH 7.2), 0.2 mL of (15 mM) H_2O_2, and 0.2 mL of enzyme extract. The reaction was stopped after 5 min by adding 3 mL of titanium reagent, which not only stopped the reaction, but also generated a yellow colored complex by interacting with the residual H_2O_2.After centrifuging the mixture for 10 min, absorbance measurements at 410 nm [40] were taken. The following formula was used to calculate catalase activity (Eq. 11).

$$CAT\ activity = \frac{\Delta\ 410}{mg\ of\ protein} \quad (11)$$

where, Δ410 represents absorbance changes measured at 410 nm immediately after combining enzyme extract with oxidants.

Preparation of titanium reagent

The 1 g of titanium oxide (TiO_2) was digested for 2 h on the heating mantle with 10 g of potassium sulphate (K_2SO_4)

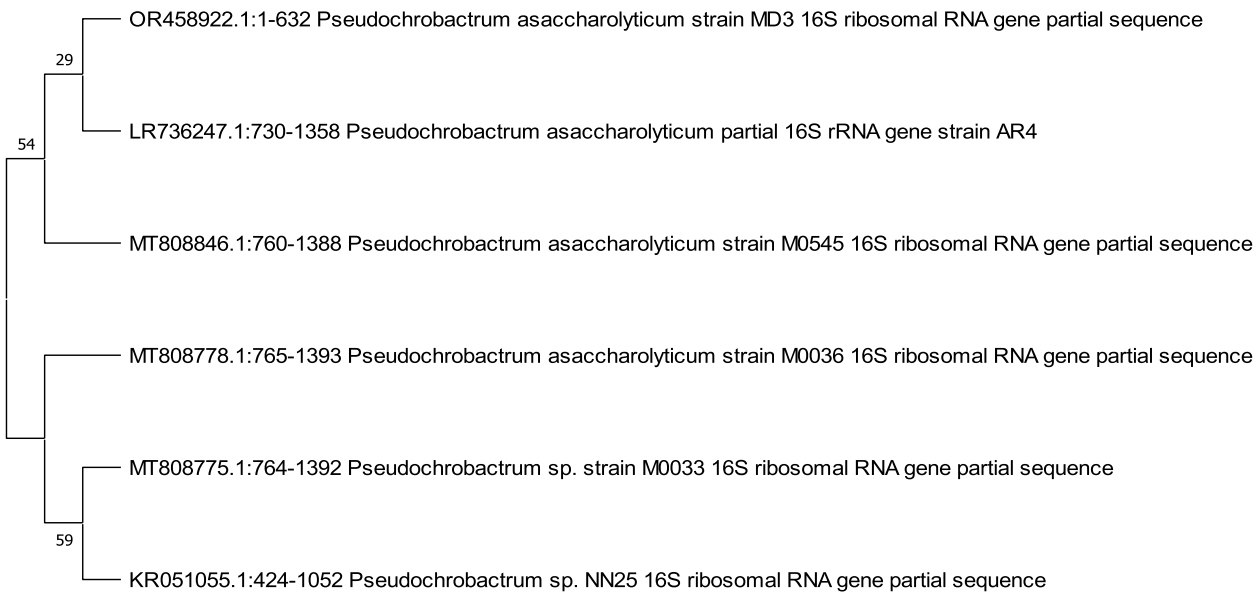

Fig. 1 Phylogenetic tree of the bacterial strain: accession No. OR458922

and 10 mL of conc. sulfuric acid (H_2SO_4) and then chilled. Diluted this to 1.5 L with distilled water before being transferred to a glass bottle [41].

c Peroxidase (POD) activity

The reaction mixture contained 0.2 mL of enzyme extract, 1.8 mL of 100 mM phosphate buffer (pH 7), 0.3 mL of 3 mM H_2O_2, and 0.1 mL of 1% w/v phenylenediamine aqueous solution. Immediately after mixing, absorbance changes were measured at 485 nm for 3 min by dual beam spectrophotometer. Variation in one unit of POD at 485 nm per minute was defined as one unit of POD. This was calculated the mg protein of the samples by using protein standard curve [42, 43]. Peroxidase activity was estimated by the following equation:

$$POD\ activity = \frac{\Delta\ 485}{mg\ of\ protein} \tag{12}$$

d Determination of arsenic contents in vegetative plant parts and soil

Digestion of plant parts (shoots, roots, and grains) and soils were performed to estimate the arsenic level. In this protocol, 1g of plant material in a 25 mL conical flask was left overnight. The 5 mL of HNO_3 was mixed with 10 mL of HNO_3-$HCLO_4$ (3:1 v/v) and digested on a hot plate [44]. Arsenic level in soil and plant parts were evaluated by using an atomic absorption spectrophotometer (AAS) (Thermo Electron S series). The bioavailable arsenic contents were calculated by formula given below.

$$\text{Arsenic}\left(\text{mg kg}^{-1}\right) = \text{As in extract mg L}^{-1} - \text{As in blank \Weight of soil (g)}$$

Statistical and principal component analysis

The data collected from each treatment in triplicate was expressed as mean ± SE. All the data was statistically analyzed through analytical software Statistics (ver. 8.1, 2005) and means were compared by Least Significant Difference. The statistical analysis (variance, simple correlations and principal component analysis) was done at the significance level $p < 0.05$ using TIBCO Statistica software (version 12.0, StatSoft Inc., Palo Alto, CA, USA). For statistical studies, principal component analysis (PCA) was utilized, and Statistica software (version 13.0, StatSoft Inc., Tulsa, OK, USA) was used. The effects of treatments with As-tolerant PGPR strain MD3 on As-contaminated maize plants were described using PCA analysis. The PCA data matrix for statistical analysis of research findings had 19 columns and 12 rows. The appropriate number of primary components was determined using Cattel's criterion. The input matrix was automatically scaled, and the principal components analysis (PCA) and correlation analysis were carried out at a significance level of $p < 0.05$.

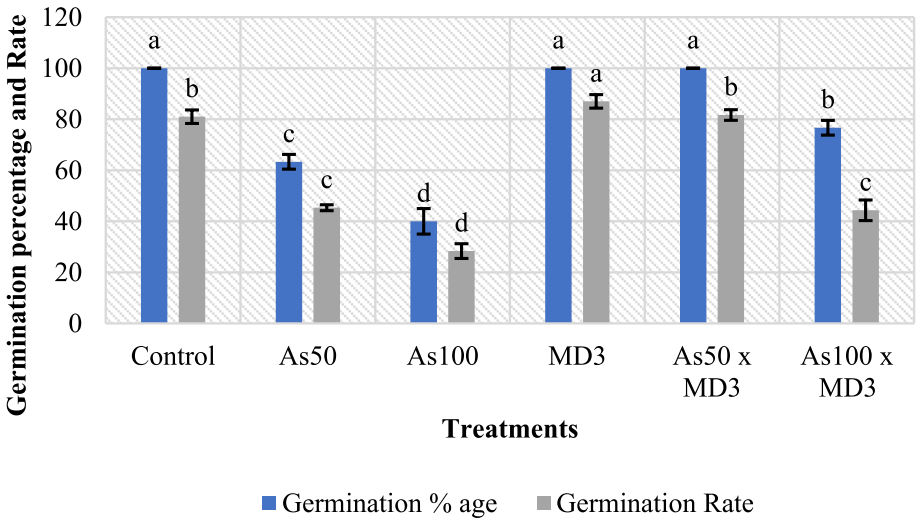

Fig. 2 Effect of PGPR strain on Germination rate and Percentage of maize seedlings under 'As' stress

Results and discussion

The results depicted that MD3 showed 96.34% similarity with *Pseudochrobactrum asaccharolyticum* and then accordingly accession number (OR458922) was allotted (Fig. 1).

Two concentrations Viz 50 mg/kg and 100 mg/kg arsenic were used because maize growth was inhibited at concentrations greater than 100 mg/kg. In preliminary test, where four concentrations 25 mg/kg, 50 mg/kg and 100 mg/kg and 150 mg/kg were used. At 150 mg/kg arsenic concentration, there was no maize growth, so, in this study we used 50 and 100 mg/kg arsenic concentrations. Arsenic induced osmotic stress, which significantly caused the strong oxidative stress and decreased the germination % age, germination rate, root and shoot length, fresh and dry weight, seedling vigor index and tolerance index by 55%, 50%, 67.28%, 75%, 35.27%, 37.28%, 37% and 35.88%, respectively. Furthermore causes and alteration in antioxidant enzymes SOD (11%), CAT (22%), POD (30%) were measured with respect to control. It also raised the MDA content by 68%. The present investigation was about studying the influence of PGPR in reducing the strength of antioxidant system thereby causing arsenic resistance in maize plant by proline and soluble sugars accumulation to elucidate the information on growth and physiological parameters.

Growth assays

Seed germination is one of the most sensitive process in metal contamination. The metabolically active cells of seeds continuously create reactive oxygen species (ROS), which appear to play significant roles in biological processes such as germination and dormancy. We investigated the effects of arsenic on seed germination and found that arsenic minimized the germination as compared to control plant. With increasing arsenic concentration, the inhibition increased.

a Germination percentage and germination rate

Results depicted that germination percentage was higher in treatments inoculated with bacterial strain than the treatments in contaminated soil without inoculation. MD3 improved the germination percentage by 25% and 22% at 50 mg/kg and 100 mg/kg arsenic levels as compared to non-inoculated treatment. Without inoculation, arsenic contamination suppressed the germination percentage up to 55% at 100 mg/kg of arsenic in soil (Fig. 2).

Germination rate was significantly decreased in treatments polluted with arsenic. Maximum reduction was noticed at 100 mg/kg of 'As,' which was around 50%. The germination rate was significantly improved by bacterial strain inoculation. Inoculation with arsenic-tolerant rhizobacteria significantly improved the germination rate by reducing the arsenic stress and improved the germination rate up to 22% and 15% at 50 mg/kg and 100 mg/kg arsenic, respectively (Fig. 2).

Germination and ROS buildup linked up, because the success of seed germination was tightly related to internal ROS contents and ROS-scavenging activities. Although ROS are dangerous molecules, but their role in cell signaling agents are now well established and widely investigated in plants. The suppression of the

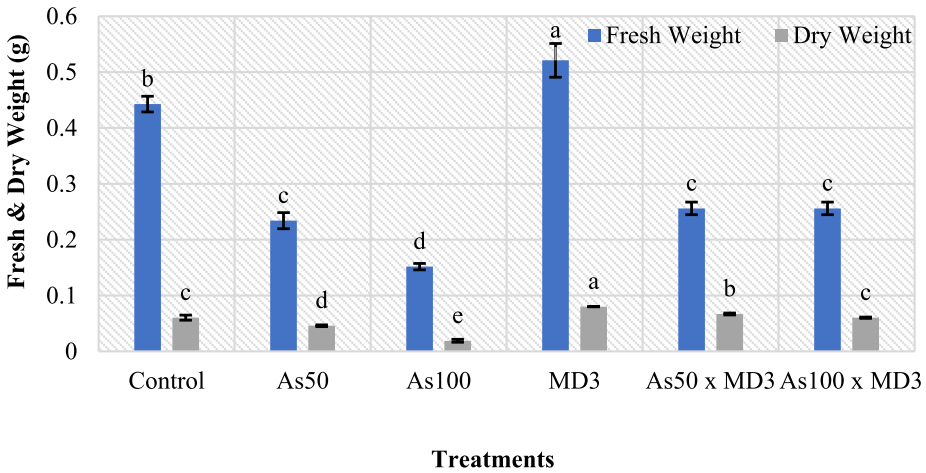

Fig. 3 Effect of PGPR strain on Fresh and Dry Biomass of maize seedlings grown under 'As' stress

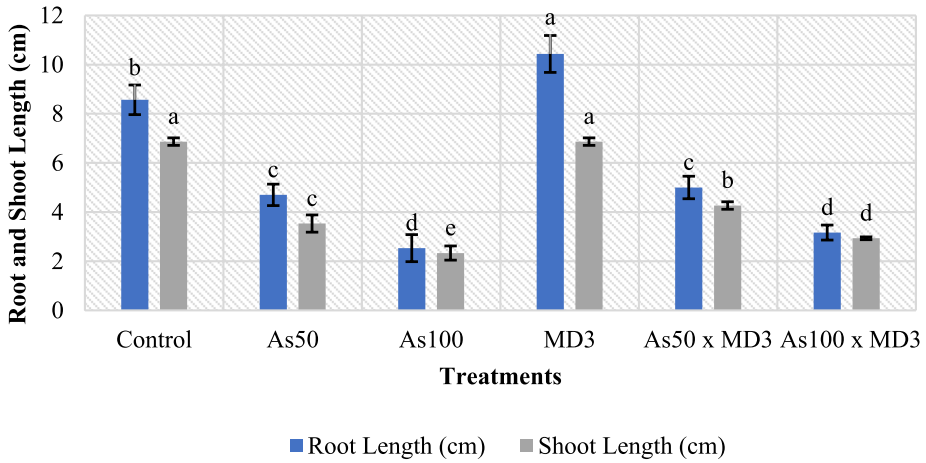

Fig. 4 Effect of PGPR strain on Root and Shoot Length (cm) of maize plants under 'As' stress

seed's physiological and metabolic activities also suppressed the germination. This decrease in germination percentage and rate corroborated with the findings, where arsenic stress severely reduced the germination percentage in *Ricinus communis* and *Pisum sativum* [45]. In our study, PGPR treated plants significantly improved the germination percentage and rate as compared to treatments of arsenic without inoculation. PGPR help in the synthesis of antibiotics, production of metabolites (siderophores), and other related processes e.g., phosphate solubilization in soil and root colonization, which boosts nutrients absorption and protection against phytopathogens. All these promote the seed germination and therefore a sustainable alternative approach for agricultural production [46].

b Fresh and dry weight

Arsenic stress on maize plants resulted a significant ($p < 0.05$) reduction in fresh and dry weight of the seedlings with respect to control (without metal stress or inoculation). The plants fresh and dry weight decreased up to 58.27% and 65.54%, respectively at 100 mg/kg of 'As' (Fig. 3). The plants grown under stress without inoculation, plants with arsenic-tolerant rhizobacteria showed a substantial ($p < 0.05$) increase in fresh and dry weights at both levels of arsenic contamination. MD3 increased the fresh and dry weights, when compared the plants grown under the same stress conditions without inoculation.

The application of arsenic caused phytotoxic symptoms as well as significant reduction in plants growth and biomass [47]. Low biomass in 'As' stress may resulted to increase the permeability of cell membrane, which in turn increased the leakage of cellular components and basic nutrients important for energy production, growth, and

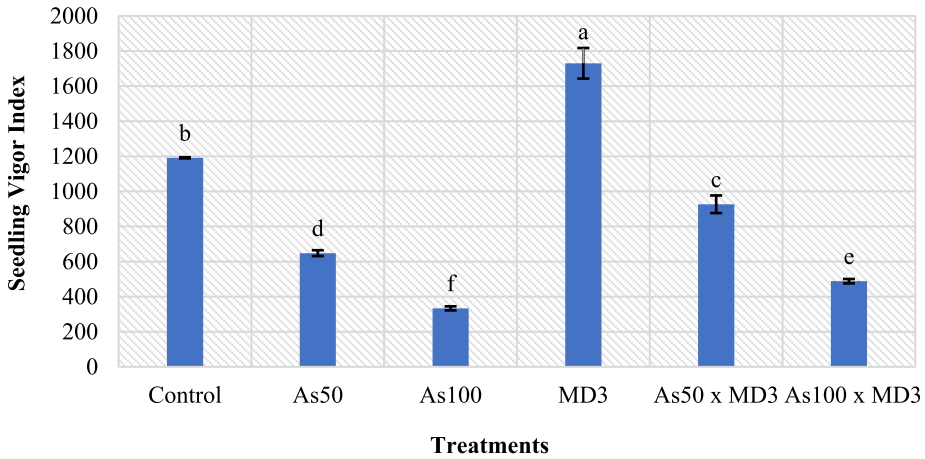

Fig. 5 Effects of PGPR strains on Seedling Vigor Index of maize seedlings under 'As' stress

development of plants [48]. In the present study, bacterial strain MD3 had significant effect on Fresh weight of maize seedlings supported by the literature, where noteworthy increased in dry weight of shoots and roots of *Eucalypts urophylla* inoculated with *Pseudomonas fulva* Ca. [49]. Another study revealed the maximum weight of potatoes shoots and roots inoculated with *Pseudomonas* [50].

c Root and shoot length

Arsenic exposure suppressed the root length ($p < 0.05$) up to 67.28% at contamination of 100 mg/kg 'As', when compared to the control. MD3 increased the root length by 20% under both levels of arsenic stress have shown non-significant results. At 100 mg/kg arsenic stress, shoot length reduced up to 75% as compared to control crop (which had neither inoculation nor heavy metal stress) (Fig. 4). However, inoculated arsenic tolerant rhizobacterial isolate improved the shoot length as compared to plants grown in metal stress without inoculation. At 50 mg/kg and 100 mg/kg of arsenic stress, isolate MD3 demonstrated the most promising outcomes and increased the shoot length up to 8% and 7%, respectively, when compared to control (without inoculation).

Root the first organ exposed to 'As' and it was observed that 'As' inhibited the root growth and expansion [51]. Plants accumulate trivalent (AsIII) and pentavalent (AsIV) arsenic. These forms interact with sulfhydryl groups on proteins and interfere with phosphate of many metabolic processes [52]. Roots are reported to be more affected than shoots, because of direct 'As' contact. In the present study, *Pseudochrobactrum asaccharolyticum* strain (MD3) treated plants showed increase in root length indicated the tolerance of MD3 strain. Similar results are reported earlier in rice, where arsenic resistant *Bacillus sp.* AsSP9 enhanced the root length [53].

d Seedling vigor index

Arsenic stress considerably ($p < 0.05$) reduced seedling vigor index, when compared to plants grown under normal conditions without arsenic and inoculation (control). Inoculation of arsenic tolerant rhizobacteria, increased the seedling vigor index and also supported the plants reversal from harmful effect of 'As'. MD3 increased the seedling vigor index by 15% and 11% at 50 mg/kg and 100 mg/kg of arsenic, as compared to plants grown under the same stress without inoculation (Fig. 5).

Because the vigor index of growth is influenced by root length, shoot length, and germination percentage at maximum arsenic concentrations. Production of root nodule and vigor index decreased due to 'As' exposure also caused wilting, curling, and necrosis of leaf blades. As previously stated, that PGPR strains are more effective in boosting vigor index, root, and shoot growth throughout the early stages of seed germination and development.

e Tolerance index

Arsenic in soil can disrupt the normal plant metabolism resulting a stunted growth and minimum crop productivity. Tolerance Index was dramatically lowered in arsenic-contaminated soils. The reduction (55.88%) was recorded at 100 mg/kg of 'As'. Inoculation with bacterial strain considerably enhanced the tolerance index. The strain MD3 performed effectively in stress reduction and improved tolerance index (11% and 8%) at 50 mg/kg and 100 mg/kg of arsenic, respectively (Fig. 6).

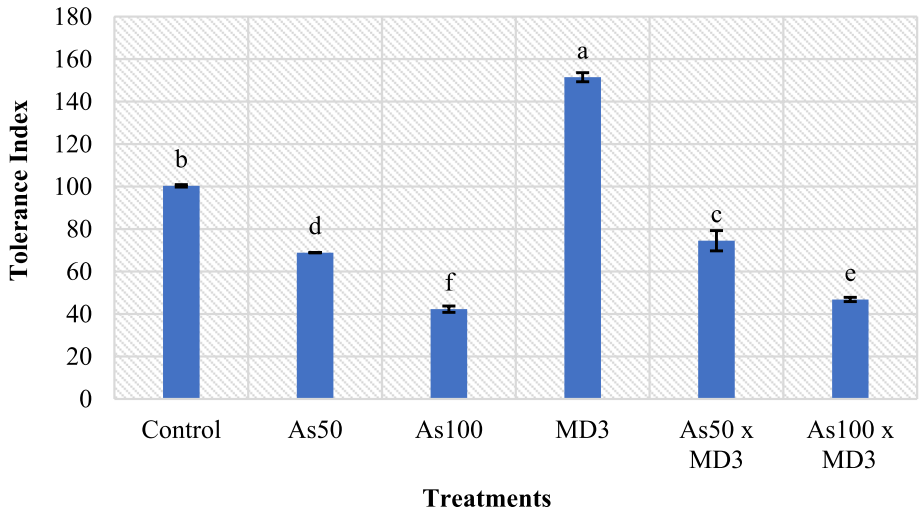

Fig. 6 Effect of PGPR strains on Tolerance Index of maize seedlings under 'As' stress

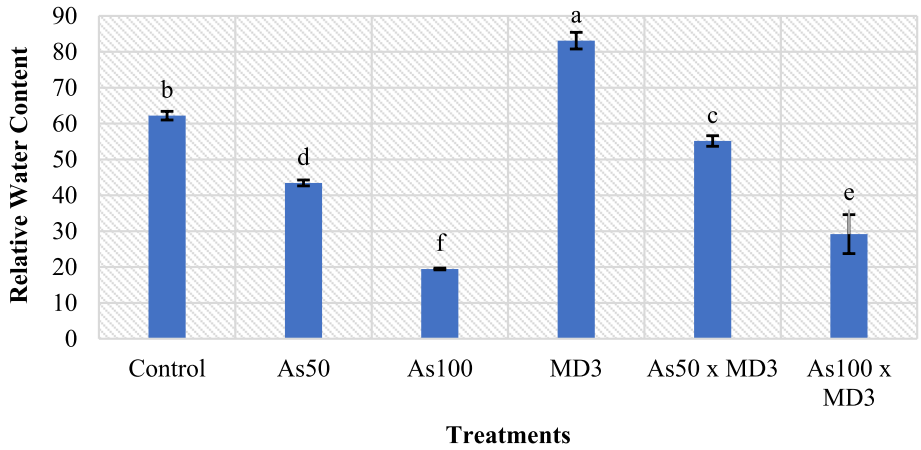

Fig. 7 Effect of PGPR strain on Relative Water Content of maize seedlings under 'As' stress

Physio-biochemical parameters

Heavy metal stress adversely affected the physiology and growth of plant, whilst inoculation with metal-resistant PGP (*Pseudochrobactrum asaccharolyticum*) reduced the arsenic pollution and increased the growth of maize seedlings.

a Relative water content

Arsenic-stress significantly lowered the RWC. Under normal conditions, RWC of non-inoculated control plants was 12% lower than plants inoculated with arsenic tolerant bacteria. Under arsenic stress, RWC of non-inoculated control plants was 28% and 35% lower than plants inoculated with MD3 (*Pseudochrobactrum asaccharolyticum*). This strain exhibited the best performance in stress reduction with 30% improved RWC at 50 mg/kg of arsenic (Fig. 7).

Arsenic stress modulates the plant water relations. 'As' stress can cause cell wall disruption in leaves and lower the water contents in leaves. PGPR treated plants have significantly higher relative water content as compared to control. PGPR inoculation in arsenic stress exhibited higher RWC as compared non-inoculated arsenic stress. Bacteria synthesize phytohormones and IAA, which assist the plant in maximum relative water content. Inoculated *T. aestivum* with *Azospirillum* sp. significantly raised the relative water content due to bacterial IAA production [54].

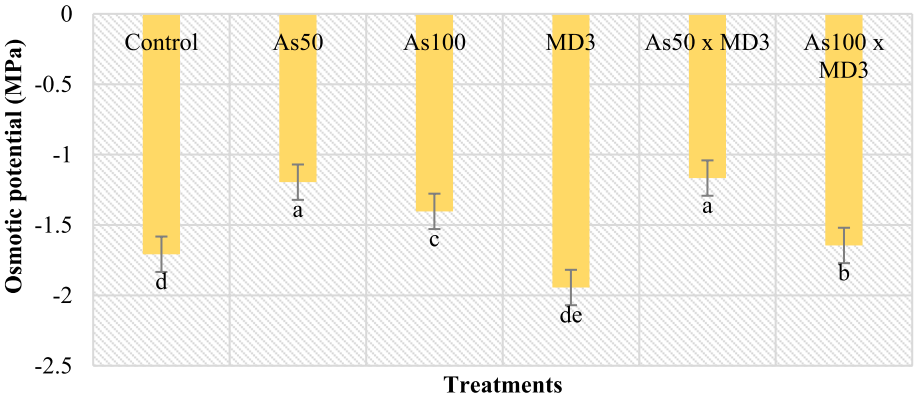

Fig. 8 Effect of PGPR strain on Osmotic Potential of maize seedlings under 'As' stress

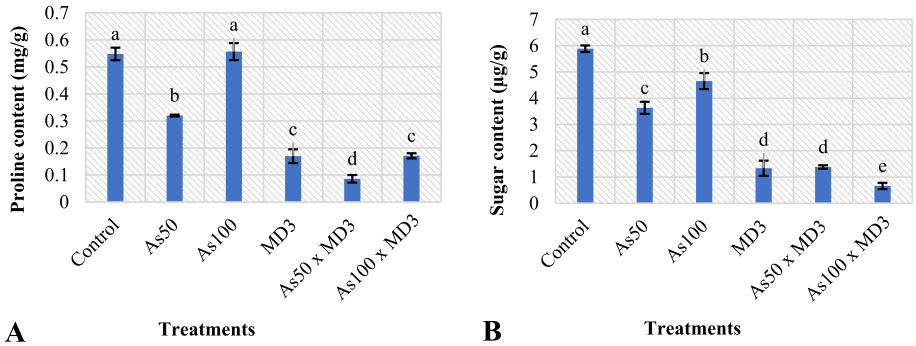

Fig. 9 Effect of PGPR strain on Proline (**A**) and Sugar (**B**) contents of maize seedlings under 'As' stress

b Arsenic induced osmotic stress

The seed treatment with bacterial strain had significant effect on the osmotic potential (leaf) of maize plants under arsenic level (100 mg/kg) of non-inoculated plants by 0.8 MPa. Inoculated plants showed an approximately drop of 0.1 to 0.3 MPa in leaf osmotic potential, when compared to control (Fig. 8).

Plants produce ROS e.g., H_2O_2, hydroxyl radicals, and superoxide anion under osmotic stress, which are hazardous to plant growth due to their deleterious effects on the plant's subcellular components and metabolism, resulting in the oxidative demise of cells. Active oxygen species degrade the membrane lipids, increased MDA, and solute leakage via membranes [55]. PGPR activates the antioxidant mechanisms beneficial in plant performance and important in relieving from osmotic stress damages by eliminating the excessive ROS and lipid peroxidation [56].

c Sugar and proline contents

Sugar and proline are antioxidants that build in higher plants in response to stress [57]. The proline content of the leaves was considerably affected by the heavy metal stress and maximize the water availability in water deficient soil. We examined the proline content of leaves from PGPR-inoculated and non-inoculated maize plants grown under normal and arsenic stress conditions. Plants exposed to arsenic accumulated higher proline (Fig. 9A) and sugar (Fig. 9B) (45% and 38%, respectively) contents. The PGPR-MD3 strain utilized in this investigation also shown the excellent effects in leaves proline content, when compared to control treatment.

Proline is also a component of non-specific defense mechanisms against heavy metal contamination and essential for plant osmoregulatory function [58]. Proline is a molecular chaperone increase the activity of

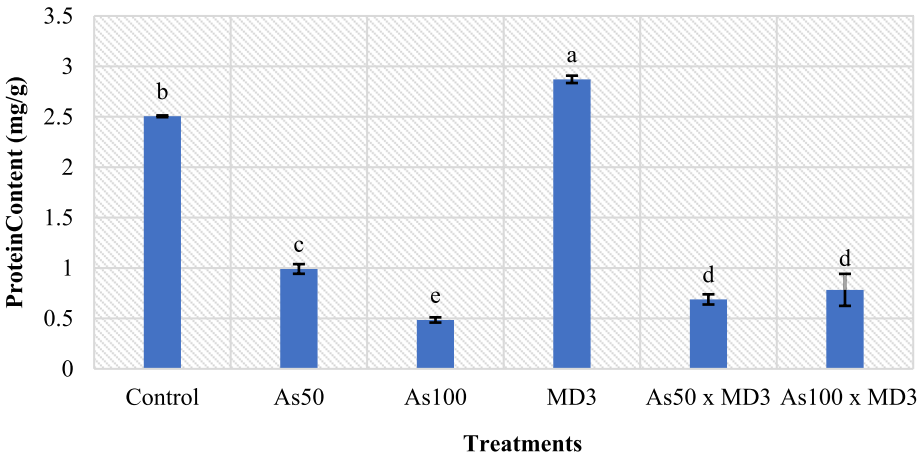

Fig. 10 Effect of PGPR strain on Protein Content (mg/g) of maize seedlings under 'As' stress

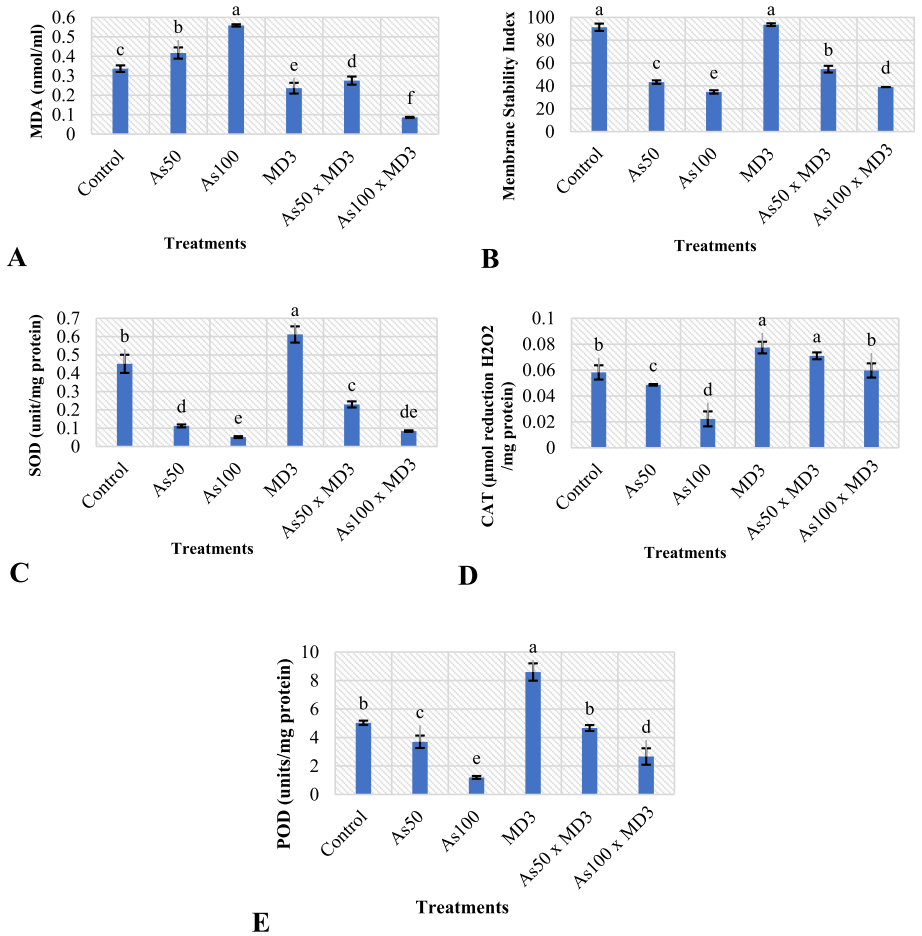

Fig. 11 Effect of PGPR strain on MDA, MSI and antioxidant activities of maize seedlings under 'As' stress

several enzymes, and safeguard the protein integrity. In our investigation, treated plants showed greater proline content during arsenic stress as compared to non-inoculated plants. Salt tolerance seen in *Zea mays* co-inoculated with *Rhizobium* and *Pseudomonas* due to increased proline production, decreased electrolyte

Table 3 Arsenic contents in plant parts and soil

Characteristics	Shoots (ppm)	Roots (ppm)	Grains (ppm)	Soil (ppm)
Control	0.045	0.058	0.031	4.866
50 ppm As	19.35	20.65	14.45	0.55
100ppm As	20.91	25.52	16.73	41.84
50pm As X MD3 Inoculation	12.55	13.55	11	17.9
100pm As X MD3 Inoculation	9	15	5	76

leakage, maintain relative water content of leaves, and selective uptake of K ions [59].

d Protein content

Arsenic drastically reduced the protein content upto 60% as compared to control plants. The results demonstrated that arsenic inhibited the production of protein content. MD3 strain notably ($p < 0.05$) improved the plant growth (Fig. 10). Protein breakdown is an adaptation of the cells in condition of sugar shortage. Plant's cell soluble protein concentration is an indicator of their physiological status. The minimum amount of soluble protein in arsenic-treated maize seedlings provoked the faster catabolism processes. The rapid catabolism is most likely due to significant disruptions in the membrane systems due to arsenic phytotoxicity, while PGPR inoculation under arsenic stress shown non-significant results.

e MDA, MSI SOD, CAT and POD activities in leaves

Arsenic at both concentrations significantly damaged the growth of maize plants, whereas the damage was severe at higher 'As' concentration. At 100 mg/kg of 'As', higher MDA and lower MSI noticed, which were improved by the addition of *Pseudochrobactrum asaccharolyticum*. MDA content was increased by 45% and 68% at 50 mg/kg and 100 mg/kg 'As' concentrations, respectively (Fig. 11A). However, as compared to control, MD3 strain reduced MDA by 20%. A 17% and 25% drop in MSI was detected at 50 mg/kg and 100 mg/kg 'As' as compared to the respective control (Fig. 11B). *Pseudochrobactrum asaccharolyticum* inoculation improved SOD, CAT, and POD activities in maize grown under 'As' contaminated soil. The SOD, CAT, and POD activities in leaves are decreased with continuous increasing level of 'As' stress. Arsenic at 100 mg/kg reduced SOD activity by 11%, CAT by 22%, and POD by 38% as compared to respective control. Contrarily, MD3 strain elevated the SOD (Fig. 11C), CAT (Fig. 11D), and POD (Fig. 11E) activities when compared to respective control. The activity of these antioxidant enzymes was highest at 'As' 50 mg/kg inoculated with *P. asaccharolyticum.*

Heavy metal exposure causes severe oxidative stress in plants, changes in antioxidant enzyme activity depending on plant species, growth stage, metal dose, and duration [60]. A few results suggested that heavy metal stress increase the oxidative stress via overproduction of ROS and MDA contents [61]. Without the addition of 'As' tolerant strains, our data showed an increase in MDA content and decreased activity of antioxidant enzymes (SOD, CAT, and POD) and MSI in maize under higher 'As' concentrations. Arsenic block the complex I of the mitochondrial electron transport chain, resulting in excessive ROS production, lipid peroxidation, protein degradation, and establishment of mitochondrial permeability transition (MPT) [62]. These modifications severely disturb the usual physiological pathways of a plant with low yield and growth. Seed inoculated with *P. asaccharolyticum*, dramatically reduced oxidative stress induced by 'As' in maize via lower MDA, higher MSI, and efficient activities of antioxidant enzyme. PGPR improved the antioxidant defense system, increased nitrogen and mineral nutrition uptake, and decreased the oxidative damage and heavy metal uptake in plants under heavy metal stress [63]. Co-inoculated *Sinorhizobium* and *Agrobacterium* proficiently increased the SOD, CAT, and APX activities in alfalfa under heavy metal stress, which generated acidic exopolysaccharides (a diffusion barrier against ROS). Lower MDA and higher antioxidant enzyme activities in wheat indicated that 'As' tolerant strain *P. asaccharolyticum* minimized the oxidative stress in plants, possibly due to lower 'As' uptake.

f Arsenic contents in plant parts and soil

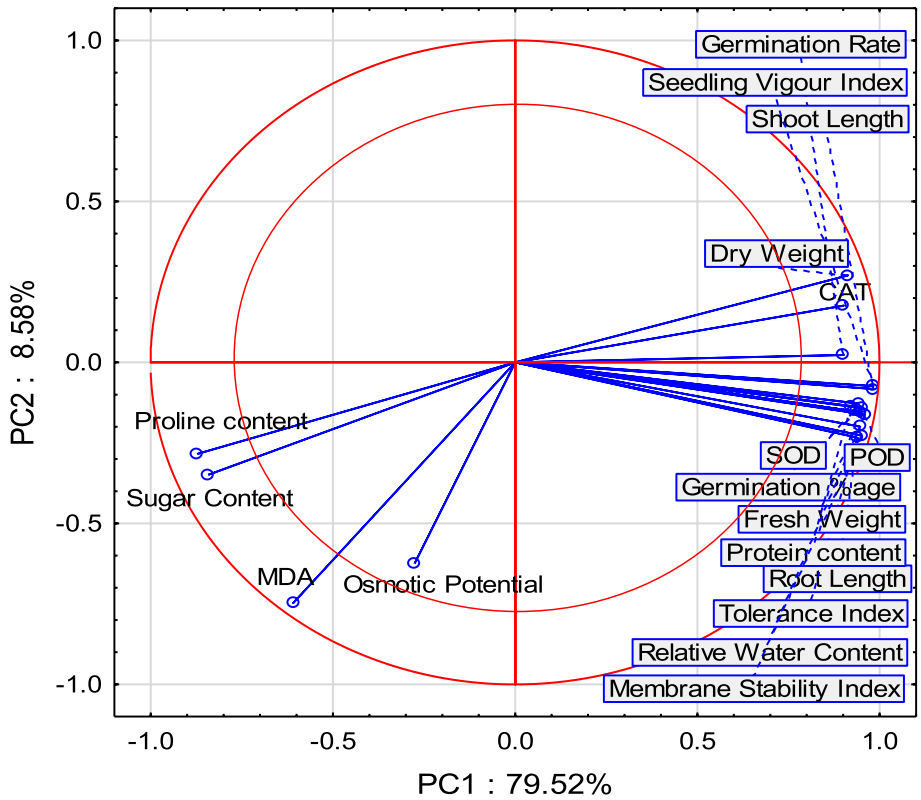

Fig. 12 Principal component analysis of the effect of As-tolerant strains inoculation on growth and physiological parameters of maize plant under arsenic stress; Loading plot of PC1 and PC2

Arsenic contents in plant parts and soils were measured (Table 3). Significant levels of arsenic were accumulated by maize plants under stress of sodium arsenate in shoots, roots, grains, and soil. Data showed that rhizobacteria inoculation immobilized the arsenic in soil, which significantly hindered the plant's ability to absorb it. Arsenic contents were lower in MD3 inoculated treatments than non-inoculated control of shoots, roots and grains.

PCA, correlation matrix and Heat map

The result of PCA analysis presented 11 new variables, and the first two principal components described as much as 88.09% variability of the system. PC1 described the largest percentage 79.52% of the variability, while the second main component PC2 described 8.58% ovariability of the system (Fig. 12). All parameters except osmotic potential have a large impact on variability of the system, because these are placed in between the two red circles. A strong positive correlation was found between sugar and proline content, whereas slightly weaker one between sugar, proline, and MDA. There was also a strong positive correlation between germination percentage, germination rate, root length, shoot length, seedling vigor index, tolerance index, fresh weight, dry weight, protein content, membrane stability index, CAT, SOD, and POD. There was a strong negative correlation between sugar content, proline content and MDA content. The performed PCA analysis shown positive values of the first principal component PC1, which explained 79.52% variability of the system.

The correlation matrix of the tested parameters were depicted in this study (Table 4). The collinearity (correlation) of the explanatory variables were represented by the determinant of the correlation matrix. The lower the degree of mutual correlation of explanatory factors, the closer to 0. The stronger the connection closer this to one. This determined whether the association was positive or negative.

The As100 and As50 were highly correlated with sugar content, proline content and MDA content, and MD3 was strongly correlated with germination percentage, germination rate, root length, shoot length,

Table 4 Correlation matrix for the tested parameters

r>=	-1	-0,80	-0,60	-0,40	-0,20	0	0,20	0,40	0,60	0,80	1

Variables	Germination percentage	Germination Rate	Root Length	Shoot Length	Seedling Vigour Index	Tolerance Index
Germination percentage	1,000000	0,767473	0,926550	0,911599	0,921122	0,926672
Germination Rate	0,767473	1,000000	0,799308	0,916869	0,911180	0,791275
Root Length	0,926550	0,799308	1,000000	0,960255	0,970902	0,998527
Shoot Length	0,911599	0,916869	0,960255	1,000000	0,989808	0,951965
Seedling Vigour Index	0,921122	0,911180	0,970902	0,989808	1,000000	0,966500
Tolerance Index	0,926672	0,791275	0,998527	0,951965	0,966500	1,000000
Fresh Weight	0,999306	0,764743	0,921143	0,909325	0,915666	0,921130
Dry Weight	0,783293	0,899464	0,770476	0,885873	0,877113	0,751296
Sugar Content	-0,701795	-0,896002	-0,645165	-0,804331	-0,794772	-0,628227
Proline content	-0,790115	-0,825428	-0,686510	-0,814489	-0,795987	-0,668581
Protein content	0,866570	0,849880	0,874670	0,928226	0,901974	0,867474
RWC	0,922638	0,874962	0,940885	0,950326	0,956408	0,942479
MSI	0,954320	0,813465	0,972611	0,953624	0,960957	0,970836
SOD	0,990497	0,719080	0,923602	0,897760	0,902947	0,923000
CAT	0,813272	0,855496	0,748003	0,856817	0,839785	0,728075
POD	0,903508	0,825117	0,960669	0,957041	0,949917	0,956577
MDA	-0,481753	-0,529118	-0,461988	-0,533705	-0,545465	-0,432695
Osmotic Potential	-0,263587	-0,077024	-0,281603	-0,225737	-0,267099	-0,261377

Variables	Fresh Weight	Dry Weight	Sugar Content	Proline content	Protein content	Relative Water Content
Germination percentage	0,999306	0,783293	-0,70179	-0,79011	0,866570	0,922638
Germination Rate	0,764743	0,899464	-0,896002	-0,825428	0,849880	0,874962
Root Length	0,921143	0,770476	-0,645165	-0,686510	0,874670	0,940885
Shoot Length	0,909325	0,885873	-0,804331	-0,814489	0,928226	0,950326
Seedling Vigour Index	0,915666	0,877113	-0,794772	-0,795987	0,901974	0,956408
Tolerance Index	0,921130	0,751296	-0,628227	-0,668581	0,867474	0,942479
Fresh Weight	1,000000	0,781150	-0,697451	-0,789438	0,865955	0,917379
Dry Weight	0,781150	1,000000	-0,950363	-0,951139	0,810464	0,793121
Sugar Content	-0,697451	-0,950363	1,000000	0,958558	-0,731732	-0,74365
Proline content	-0,789438	-0,951139	0,958558	1,000000	-0,757892	-0,76127
Protein content	0,865955	0,810464	-0,731732	-0,757892	1,000000	0,914667
RWC	0,917379	0,793121	-0,743655	-0,761270	0,914667	1,000000
Membrane Stability Index	0,947017	0,778366	-0,706614	-0,753152	0,896131	0,967965
SOD	0,992033	0,753350	-0,649282	-0,747714	0,853234	0,886869
CAT	0,813128	0,941317	-0,914419	-0,946979	0,827830	0,839032
POD	0,902743	0,809824	-0,686151	-0,728887	0,870889	0,891016
MDA	-0,466925	-0,718976	0,706294	0,690175	-0,465896	-0,41237
Osmotic Potential	-0,245755	-0,243111	0,202468	0,186938	-0,145339	-0,10550

Variables	Membrane Stability Index	SOD	CAT	POD	MDA	Osmotic Potential
Germination percentage	0,954320	0,990497	0,813272	0,903508	-0,481753	-0,26358
Germination Rate	0,813465	0,719080	0,855496	0,825117	-0,529118	-0,07702
Root Length	0,972611	0,923602	0,748003	0,960669	-0,461988	-0,281603
Shoot Length	0,953624	0,897760	0,856817	0,957041	-0,533705	-0,225737
Seedling Vigour Index	0,960957	0,902947	0,839785	0,949917	-0,545465	-0,267099
Tolerance Index	0,970836	0,923000	0,728075	0,956577	-0,432695	-0,261377
Fresh Weight	0,947017	0,992033	0,813128	0,902743	-0,466925	-0,245755
Dry Weight	0,778366	0,753350	0,941317	0,809824	-0,718976	-0,243111
Sugar Content	-0,706614	-0,649282	-0,914419	-0,686151	0,706294	0,202468
Proline content	-0,753152	-0,747714	-0,946979	-0,728887	0,690175	0,186938
Protein content	0,896131	0,853234	0,827830	0,870889	-0,465896	-0,145339
Relative Water Content	0,967965	0,886869	0,839032	0,891016	-0,412378	-0,105500
Membrane Stability Index	1,000000	0,934325	0,795803	0,918620	-0,480637	-0,276855
SOD	0,934325	1,000000	0,773635	0,903066	-0,460409	-0,309603
CAT	0,795803	0,773635	1,000000	0,745400	-0,631029	-0,155339
POD	0,918620	0,903066	0,745400	1,000000	-0,533281	-0,243252
MDA	-0,480637	-0,460409	-0,631029	-0,533281	1,000000	0,694722
Osmotic Potential	-0,276855	-0,309603	-0,155339	-0,243252	0,694722	1,000000

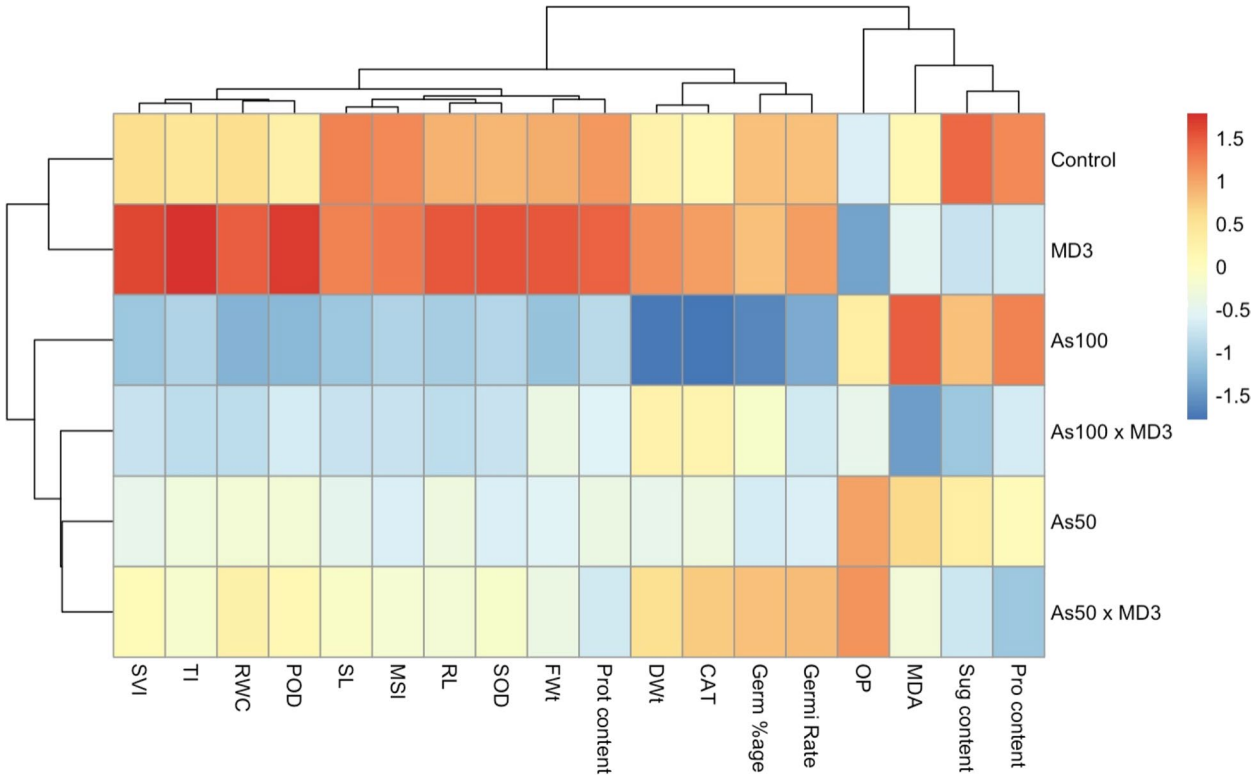

Fig. 13 Heatmap indicated the effect of Arsenic stress and As-tolerant strains inoculation on growth and physiological parameters of maize plant

seedling vigour index, tolerance index, fresh weight, dry weight, protein content, membrane stability index, CAT, SOD, and POD.

The heatmap revealed that treatments with As-tolerant strain MD3 was positively correlated with maize plant growth and physiological parameters (Fig. 13), and these treatments were more isolated than all other treatments, indicating significant differences in inoculated and non-inoculated maize plants.

Conclusion

Heavy metals stress is a big hindrance in sustainable agriculture, crop production, quality, and yield. Arsenic toxicity triggers osmotic imbalances, photo oxidative damages of proteins, DNA, lipids, and eventually the plants cell death. ROS in plant cells display detrimental effects on plant growth and development. Our findings revealed significant improvement by PGPR inoculation at germination and physiological level in arsenic effected plant, because As-tolerant strain (*Pseudochrobactrum asaccharolyticum*) improved the growth by proline production, which reduced in shoots and roots of target plant. Proline is an antioxidative defense molecule that scavenges ROS, and signaling a particular gene that recover the plants from heavy metals stress. Furthermore, bacterial inoculation increased the activities of antioxidants e.g., SOD, CAT, and POD, which scavenge the excessive ROS, indicating better stress management in plants (Fig. 14).

The current study concluded the active participation *P. asaccharolyticum*, which maximize the 'As' tolerance and removal by indirect activation of protective defense mechanisms for better plant health. However, further studies are still needed to evaluate the mechanisms at molecular level. This strain would be a new candidate for potential application in different metals pollution and bioremediation of contaminated soils. The co-occurrence of heavy metals and chemical fertilizers in agricultural settings raise the significant concerns for public health. This study further draws attention to the increasing dual resistance of bacteria towards heavy metals and better plant growth, which may fight against anti microbes in agricultural settings. The consequences of these results will increase the awareness to find the other bacterial strains, which act against metal toxicity and improve the quality of food crops. Stricter regulations, monitoring,

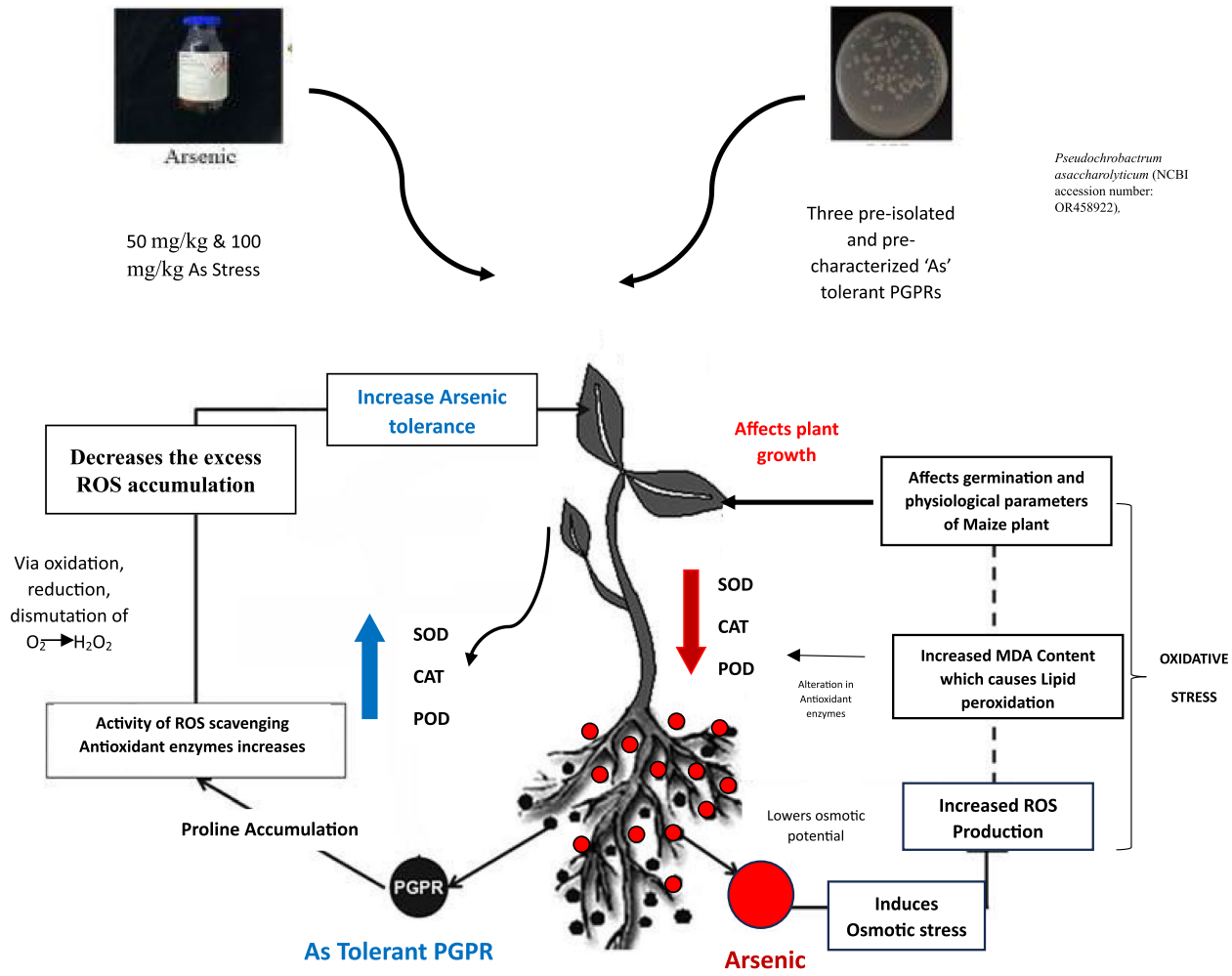

Fig. 14 Arsenic tolerant PGPR ameliorates Arsenic induced oxidative stress

reduction in use of chemical fertilizers, and adoption of bio fertilizers with sustainable farming practices are still desirable. On behalf of successful results of this study, using microorganisms to mitigate pollution and nowadays eco-friendly bioremediation approaches are considering pragmatic over physicochemical processes.

Abbreviations

A	Absorbance
PGPR	Plant growth promoting rhizobacteria
PCA	Principle component analysis
CAT	Catalase
MLND	Maize lethal necrosis disease
E	Transpiration rate
EDTA	Ethylenediaminetetraacetic acid
FYM	Farmyard manure
g	Gram
H_2O_2	Hydrogen peroxide
IRGA	Infra-red gas analyzer
IU	Enzyme activity
K	Kelvin
Kg	Kilogram
MA	Molar absorption
µL	Microliter
µmol	Micromole
mbars	Millibars
MDA	Melondialdehyde
mL	Milliliter
mm	Millimeter
mM	Millimolar
Mol	Mole
MPa	Megapascal
nm	Nanometer
NPK	Nitrogen, phosphorus and potassium
PAR	Photosynthetically active radiation
PCA	Principal component analysis
PM	Press mud
P_{net}	Net photosynthetic rate
POD	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
MSI	Membrane stability index
T	Temperature
TBARS	Thiobarbituric acid reactive substances
UV–vis	Ultra violet-visible
v/v	Volume per volume
WUE	Water use efficiency
w/v	Weight per volume
IU	International unit of enzyme activity

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Authors' contributions
ZW, SI, KJ, MI designed the experiments. ZW performed the experiments. SI, KJ AU, RMA analyzed and interpreted the data. ZW, MI, AU, SMA wrote and reviewed the manuscript. RMA, SMA, SI, AU commented on the manuscript. SI, AU, RMA, MG reviewed and addressed the revision comments with their expertise. All authors read and approved the final version.

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Competing interests
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