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Research article

Pseudomonas citronellolis; a multi-metal resistant and potential plant growth promoter against arsenic (V) stress in chickpea

Arindam Adhikary[a], Rajiv Kumar[a], Ranjna Pandir[a], Pankaj Bhardwaj[b], Ramakrishna Wusirika[c], Sanjeev Kumar[a,b,*]

[a] Centre for Biosciences, School of Basic and Applied Sciences, Central University of Punjab, Bathinda, 151001, India
[b] Department of Plant Sciences, School of Basic and Applied Sciences, Central University of Punjab, Bathinda, 151001, India
[c] Department of Biochemistry and Microbial Sciences, School of Basic and Applied Sciences, Central University of Punjab, Bathinda, 151001, India

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ABSTRACT

Soil micro-biota plays a vital role in maintaining plant growth and fitness under normal and adverse conditions. *Pseudomonas* is one of the most important free-living and copious genera in south-west Punjab and involved in plant growth promotion under heavy metal stress. In this study, we have studied microbial diversity of the agricultural and marginal land based on 16S rRNA gene and screened eight strains of *Pseudomonas* for its tolerances towards various heavy metals and for plant growth promoting properties (PGP). The best strain is tested in chickpea plants against Arsenic (As^{5+}) stress. All the strains responded differently to heavy metals *viz.* Arsenic, (As^{5+} (0.3–0.5M) and As^{3+} (250 μg mL^{-1}) Cadmium (Cd^{2+}) (250–350 μg mL^{-1}), Chromium (Cr^{2+}) (200–350 μg mL^{-1}) and Mercury (Hg^{2+}) (1–2 μg mL^{-1}). Out of eight strains, only two strains (KM594398 and KM594397) showed plant growth promoting characters, concurrently they were highly tolerant to Arsenic (As^{5+}). *Pseudomonas citronellolis* (PC) (KM594397) showed the best results in terms of As^{5+} tolerance and plant growth promoting activity, hence further tested for actual plant growth response in chickpea (*Cicer arietinum* L.) under As^{5+} (10–160 mg kg^{-1}) stress. *Pseudomonas citronellolis* enhanced plant growth and dry biomass under As^{5+} stress. High As^{5+} tolerance and plant growth promoting activity of *Pseudomonas citronellolis* in chickpea especially designate this strain suitable for marginal lands and heavy metals contaminated sites.

1. Introduction

Heavy metals are naturally present in the environment and some of these heavy metals play an important role in various metabolic functions in the living organisms. These heavy metals and metalloids *viz.* As^{5+}, As^{3+}, Cd^{2+}, Cr^{2+}, and Hg^{2+} are non-essential for living organisms and are toxic to the cells even at minute concentration. Among these heavy metals and metalloids, Arsenic is the most toxic metalloid (ATSDR). It mimics phosphate ion and can easily penetrate the cell membrane. Cytoplasmic As^{5+} gets converted to As^{3+} by Arsenate reductase, further As^{3+} reacts with sulfhydryl groups (–SH) of enzymes and tissue proteins causing disruption of cellular function and even cell death (Smith et al., 2010). Cadmium (Cd^{2+}), a divalent cation is associated with chlorosis, necrosis, nutrient imbalance, oxidation of proteins, lipids, and nucleic acids, disruption of electron transport chain and membrane bound enzymes such as NADPH oxidases (Khanna et al., 2019). Chromium (Cr^{2+}) and mercury (Hg^{2+}) are highly toxic to plants. Hg^{2+} interferes with mitochondrial activity and induces oxidative stress (Nagajyoti et al., 2010). Chromium also affects CO_2 fixation, electron transport, photophosphorylation, and enzyme activities (Nagajyoti et al., 2010). Higher concentrations of heavy metals in soil have proven to be toxic not only for plants but also for microbes. Studies have shown that the population of various microbial communities such as bacteria, fungi, their biomass, number, activity, basal respiration and substrate utilization are negatively affected by elevated heavy metal levels (Liao and Xie, 2007).

* Corresponding author. Department of Plant Sciences, School of Basic and Applied Sciences, Central University of Punjab, India.
E-mail addresses: sanjeev.kumar@cup.edu.in, sanjeevpuchd@gmail.com (S. Kumar).

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Heavy metals inhibit various metabolic processes such as primary productivity, enzyme synthesis, nitrogen fixation and carbon, nitrogen, sulphur, and phosphorus mineralization ability of the soil microbial population (Babich and Stotzky, 1985). However, few bacteria such as *Bacillus, Pseudomonas, Serratia*, and *Acidobacter* can tolerate heavy metal contamination as they have developed adaptive and genetic systems that can counteract the effects of high levels of heavy metal ions (Trajanovska et al., 1997). Yin et al. (2015) have reported that sedimentary microbial communities adapt to heavy metal contamination by altering their compositional structure and functional priority especially the key functional genes which are involved in metal homeostasis. Generally, heavy metal resistant bacteria inhibit mobility and availability of the metals to the plants. Among the heavy metal resistant bacteria, a few bacteria possess ability to produce phytohormone (Indole acetic acid), siderophores, reduce soil pH, and/or solubilise metal phosphates (Cavalca et al., 2010) thereby, modify the efficiency of the accumulation processes. Phosphate solubilizing bacteria especially provide soluble phosphate, an essential nutrient for plant growth and development for uptake. A phytohormone, Indole Acetic Acid (IAA) that helps in the induction of root growth in plants is also produced by the heavy metal resistant bacteria (Singh, 2013). So far, several heavy metal resistant bacteria with potential plant growth promoting characteristics under heavy metal stress have been reported (Khanna et al., 2019; Li and Ramakrishna, 2011). Li and Ramakrishna (2011) reported that *Pseudomonas* isolated from the metal rich soil are resistant to multiple heavy metals (Cu^{2+}, Zn^{2+}, Cs^{+}, Hg^{2+}, As^{3+}) and promote plant growth under Cu^{2+} stress. In *Lycopersicon esculentum*, inoculation of *Pseudomonas aeruginosa* and *Burkholderia gladioli* has increased the growth and photosynthetic pigments under Cd stress (Khanna et al., 2019). Therefore, heavy metal resistant bacteria with plant growth promoting trait can be a potential candidate for plant growth promotion in heavy metal contaminated sites.

Punjab is situated in the North-West of India and mainly relies upon agriculture. Extensive use of chemical fertilizers, pesticides, and insecticides has led to heavy metal contamination in this area. South –west Punjab, which occupies around 15% of Punjab's cultivated area consumes around 75% of the total pesticides used in Punjab (Mittal et al., 2013). In a previous study (Vanita et al., 2014), Fe^{3+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+} and Ni^{2+} content were higher in the agricultural soil as compared to control soil and were significantly genotoxic to *Allium cepa.* Hence, there is a need for green initiatives to promote plant health and crop production in the heavy metal contaminated soil and use of heavy metal resistant plant growth promoting bacteria can be the possible solution.

In present study, we have analysed bacterial community status of this area, and tested eight isolated *Pseudomonas* sp. Strains from the soil against non-essential heavy metals e.g. As^{5+}, As^{3+}, Cr^{2+}, and Cd^{2+}. Lastly, we determined the performance of best plant growth promoting bacterial strain in chickpea (*Cicer arietinum* L.)

2. Materials and methods

2.1. Analysis of the bacterial community in heavy metal contaminated soil

Soil samples were collected from three different sites of Bathinda region: Central University of Punjab (30.170N, 76.450E), and two agriculture land sites (30.32290N, 74.92340E and 30.108750N, 75.0910E). At each site, soil samples from (0–10 cm depth) were randomly collected in triplicates from three different locations that are 100m apart from each other. The soil samples from each site were collected in triplicate, homogenized together, and composite samples were sieved. The samples were stored at −80 °C for extraction of bacterial DNA.

Soil microbial DNA was extracted from sieved soil samples of the three sites by method given by Ellis et al. (2003) with minor modifications. The quality of DNA was checked on 0.8% agarose gel, and the quantity of DNA was checked with Nanodrop spectrophotometer (Thermo Scientific model 2000). The 16S rRNA gene clone library was constructed using 16S rRNA gene universal primers, 27F (AGAGTTTG ATCMTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT). Each PCR mix consisted of 0.25 mM L^{-1} each of dNTP, 2 ng of each primer, 1X PCR buffer including 1.5 mM $MgCl_2$, and 1U DNA Taq polymerase (Applied Biosystems, USA), 50 ng DNA and 1.8 μL MilliQ water in a total volume of 10 μL. The following PCR conditions were used: 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min denaturation, 48 °C for 2 min of annealing and 72 °C for 1:30 min for extension; and a final extension at 72 °C for 8 min. The amplified products were cloned using TOPO-TA PCR cloning kit (Invitrogen, USA). Clones were selected by blue-white screening and colony PCR was performed to confirm the inserted fragments into the vector. Each colony PCR mix consisted: 0.25 mM L^{-1} each of dNTPs, 2 ng of each primer (M13F and M13R), and 1 X PCR buffer include 1.5 mM $MgCl_2$, and 1U DNA Taq polymerase (Applied Biosystems, USA), 1 μL colony suspension and 2.3 μL MilliQ water in a total volume of 10 μL. The following PCR conditions were used: 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s of denaturation, 55 °C for 45 s of annealing and 72 °C for 1.30 min of extension; and a final extension at 72 °C for 8 min. Positive clones were further used for plasmid DNA isolation using alkaline lysis method (Sambrook, 2001). Universal primer M13F, 515F and 895F were used to amplify the inserted sequences; and sequencing of the amplified products was done according to ABI 3730 XL DNA Analyzer (Applied Biosystems, USA) manufacturer's protocol. 16S rRNA gene fragments (M13F, 515F, and 895F) were compiled (using DNA STAR Lasergene ver. 10SeqMan) and aligned. Sequences were analysed by using GenBank database and BLAST algorithm. All the sequences obtained from the soil samples of different sites were aligned, and phylogenetic analysis was performed using the Neighbour-Joining method in MEGA 7 software (Kumar et al., 2016). For evolutionary distance computation, Jukes-Cantor method was used (Jukes and Cantor, 1969). 16S rRNA gene sequences were deposited in GenBank nucleotide database (Accession numbers JX280020 to JX280145).

Based on the phylogenetic analysis, *Pseudomonas* was found to be the most abundant genera. Then we isolated the *Pseudomonas* spp. only and characterized biochemically and validated using 16S rRNA sequencing (Adhikary et al., 2019). In this study, we have tested the isolated *Pseudomonas* strains against different heavy metals and for their PGP properties in a 45 days experiment with five varieties of chickpea and As^{5+} with the best performing strain.

2.2. Characterisation of Pseudomonas isolates

2.2.1. Pseudomonas tolerance to heavy metals and metalloid

Eight strains of *Pseudomonas* (Accession no. KM594392, KM594399, KM594393, KM594394, KM594395, KM594397, KM594396, and KM594398) were used for determination of minimum inhibitory concentration (MIC) and maximum tolerance capacity (MTC) for Sodium arsenite (As^{3+}), Sodium arsenate heptahydrate (As^{5+}), Potassium dichromate (Cr^{2+}), Mercuric chloride (Hg^{2+}) and Cadmium nitrate (Cd^{2+}) (Bhojiya and Joshi, 2016). For each metal, triplicate tubes with increasing metal concentration were prepared and 50 μL of freshly

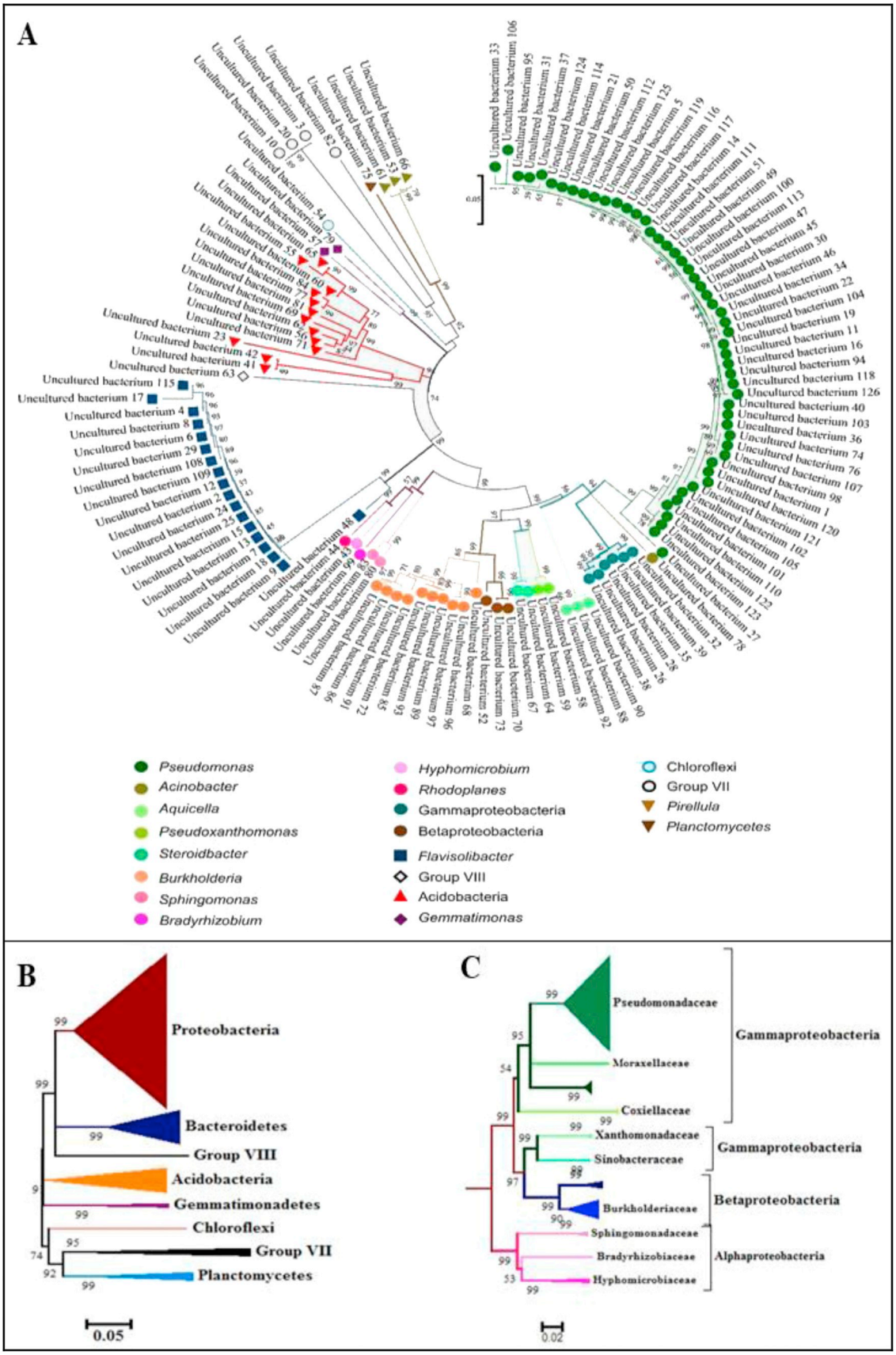

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Fig. 1. Phylogenetic analysis of 126 16S rRNA gene isolated directly from metal contaminated soil. Neighbour-end joining tree showing relationship between different microbial communities identified on the basis of 16S rRNA gene. The sequences of 126 16S rRNA clones were aligned by ClustalW. The optimal tree with the sum of branch length = 3.27 is shown. The evolutionary distances were computed using the Jukes-Cantor method. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test (500 replicates) is shown next to the branches. Evolutionary analyses were conducted in MEGA7. **1(A)** Phylogenetic tree of 16S rRNA gene colonies at Phylum level. Neighbour-end joining tree showing relationship between different 16S rRNA gene clones **(B)** Evolutionary relationship between different phyla existing in the metal contaminated soil habitat. **(C)** Phylogenetic tree showing relationship with different groups of Proteobacteria. The evolutionary distances were computed using the Jukes-Cantor method. Evolutionary analyses were conducted in MEGA7. (2 column).

grown (< 24 h) bacterial broth solution was added as inoculum. The inoculated tubes were then incubated in a shaker incubator at 37 °C and examined at intervals for three days. The concentration at which bacterial growth is completely inhibited determines the MIC and the maximum concentration at which the bacteria can grow determines the maximum tolerance capacity (MTC) of bacterial strains.

2.2.2. Quantitative estimation of indole acetic acid (IAA) production in pseudomonas isolates

Quantitative estimation of IAA production by the bacterial isolates was done according to Tsavkelova et al. (2007). Isolates were grown in 5 mL of nutrient broth amended with Tryptophan for 48 h at 37 °C. After 48 h of incubation, 2 mL of bacterial broth culture was centrifuged at 10,000 rpm for 10 min, and supernatant collected. To 1 mL of the supernatant, 2 mL of Salkwoski's reagent was added with continuous mixing in each tube. The mixtures were then placed in the dark for 30 min. Development of pink colour was assayed at 530 nm.

2.2.3. Semi-quantitative measurement of phosphate solubilisation

Pure cultures of eight *Pseudomonas* isolates were screened for their Tri-calcium Phosphate (TCP) solubilisation activity on Pikovskaya Agar Medium (PKV) plates (Verma et al., 2015). Isolates were spot inoculated on the PKV agar plates and incubated at 28 °C for seven days. A clear zone around the growing colony indicated Phosphate solubilisation and Phosphate solubilisation index (PSI) was calculated as per the formula given below.

$$PSI = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

Table 1
Maximum Tolerance Capacity (MTC) & Maximum Inhibitory Concentration (MIC) of the isolated *Pseudomonas* strains.

Isolates	Heavy metal concentrations (μg mL^{-1})					
		Cr^{2+}	Hg^{2+}	Cd^{2+}	As^{5+}	As^{3+}
KM594392 *Pseudomonas* sp. RA-4	MTC	100	**1**	250	**0.4M**	**200**
	MIC	200	**2**	300	**0.5M**	**250**
KM594393 *Pseudomonas mendocina* strain MLCCp6	MTC	200	**1**	250	**0.4M**	**200**
	MIC	**350**	**2**	300	**0.5M**	**250**
KM594394 *Pseudomonas mendocina* strain MLCCp6	MTC	200	–	**300**	**0.4M**	**200**
	MIC	300	1	**350**	**0.5M**	**250**
KM594395 *Pseudomonas* sp. 418	MTC	200	–	**300**	**0.4M**	**200**
	MIC	250	1	**350**	**0.5M**	**250**
KM594396 *Pseudomonas* sp. G1DM-23	MTC	200	**1**	200	0.2M	**200**
	MIC	250	**2**	250	0.3M	**250**
KM594397 *Pseudomonas citronellolis*	MTC	200	–	250	**0.4M**	**200**
	MIC	250	1	300	**0.5M**	**250**
KM594398 *Pseudomonas* sp. *RA-6*	MTC	200	–	250	**0.4M**	**200**
	MIC	250	1	300	**0.5M**	**250**
KM594399 *Pseudomonas mendocina* strain NaF–C-1	MTC	**250**	–	250	**0.4M**	**200**
	MIC	300	1	300	**0.5M**	**250**

*-Bold signifies highest MIC and MTC showed by the isolates in different metals.

Table 2
Plant growth promoting characteristics of the isolates.

	IAA production (μg mL^{-1})	Phosphate solubilisation index (PSI)	Catalase test
KM594392	81.9 ± 0.88	–	+
KM594393	118.9 ± 0.74	–	+
KM594394	80.8 ± 1.65	–	+
KM594395	80.1 ± 1.00	–	+
KM594396	87.4 ± 1.41	–	+
KM594397	**138.1 ± 0.67**	**2.7**	+
KM594398	126.1 ± 1.05	1.97	+
KM594399	80.4 ± 0.71	–	+

*Bold signifies highest value among the isolates.

2.3. Effect of Pseudomonas citronellolis on growth and development of chickpea under As^{5+} stress

The effect of *Pseudomonas citronellolis* (PC) isolate on growth and development of chickpea under Arsenic stress was examined in three inches diameter pots containing 230 g of heat sterilized sand. The sand was treated with distilled water for control and different concentrations of Arsenic solution and 10^{-8} CFU/mL bacterial inoculum for different treatments. The experiments were divided into two broad categories: metal treated (M) and metal + inoculum treated (M + I). Five different As^{5+} concentrations (10 mg kg^{-1}, 20 mg kg^{-1}, 40 mg kg^{-1}, 80 mg kg^{-1} and 160 mg kg^{-1}) were used in the experiment. In another category, plants without any metal treatments and inoculum were considered as control (C) plants and plants with only inoculum treatment were considered as inoculum treated plants (I). Five chickpea varieties; PBG1, PBG5, GPF2, PDG3 and PDG4 were selected for the experiment. The chickpea seeds were incubated in different Arsenic and inoculum containing solutions for 1 h before sowing in the respective sand filled pots. After germination, the pots were kept at 25 ± 2 °C and 16/8 h light/dark photoperiod in the growth chamber. The plants were grown for 45 days and supplemented with 1X Hoagland solution at intervals of every 10 days. On the 45th day, plants were harvested for different morpho-physiological analysis such as shoot length, root length, shoot dry weight, root dry weight, Electrolyte leakage index (Lutts et al., 1996), Malondialdehyde (MDA) content (Heath and Packer, 1968) and Chlorophyll content (Arnon, 1949).

2.3.1. Electrolyte leakage index

Electrolyte leakage index indicates the membrane damage in plants. 50 mg of fresh leaves was weighed, washed and kept in 10 mL of distilled water for 18 h. The conductivity of electrolyte (L_0) was noted. Then test tubes containing distilled water and leaf tissues were autoclaved at 121 °C for 20 min. Again the conductivity of the solution was noted (L_t). The percentage electrolyte leakage index (ELI%) was calculated according to the formula: $I = (L_0/L_t) \times 100$, L_0 = electrical conductivity of the sample under before boiling and L_t is electrical conductivity of the same sample after boiling (Lutts et al., 1996).

2.3.2. Estimation of malondialdehyde (MDA) content

Malondialdehyde (MDA) content was assayed according to Heath and Packer (1968). For MDA content analysis, 50 mg of fresh leaves were homogenized in 2 mL extraction buffer containing 0.1% (w/v) TCA. The homogenate was centrifuged at 15,000g for 5 min 1 mL of the supernatant was added to 4 mL of 0.5% (w/v) TBA in 20% (w/v) TCA. The mixture was then incubated at 95 °C for 30 min and the reaction was stopped by placing the samples in an ice bath. A light pink colour appeared which was read at 532 nm and a non-specific absorbance was recorded at 600 nm. The final MDA content formed was calculated by using extinction co-efficient 155 mMol-1 cm^{-1} and expressed as μM min^{-1} mg^{-1} FW.

2.3.3. Estimation of chlorophyll content

For estimation of chlorophyll, 50 mg of fresh leaves were homogenized in 2 mL of 80% acetone and incubated at 4 °C for overnight. The homogenate was centrifuged at 5000 rpm for 5mins and absorbance was recorded at 645 nm and 663 nm. The amount of chlorophyll was calculated according to Arnon (1949) and expressed as μg/mg FW.

Chlorophyll *a* = 12.7 (A663) - 2.69 (A645)
Chlorophyll *b* = 22.9 (A645) - 4.68 (A663)

2.4. Statistical analysis

The physiological experimental data was subjected to a two-way ANOVA (analysis of variance) using Sigma Plot 11.0 software and all pair-wise comparisons of the mean responses to the different treatment groups were performed by Tukey's Test at $p < 0.05$.

3. Results

3.1. Bacterial community analysis of heavy metal contaminated soil

16S rRNA gene clone libraries from the soil samples of three different locations were constructed. From these libraries, 366 white colonies were picked and subcultured on LB agar plates. The colony PCR of the subcultured clones showed 126 positive clones with ~1700bp product. Plasmids were isolated, and M13F, 515F, and 895F were used for genome walking to cover the whole ~1500 bp of the 16S rRNA gene. The sequencing information was generated for all the 126 clones with around 1500 bp for 54 clones and around 925 bp for 72 samples. All the sequenced fragments were clustered, and chimeras were removed by SeqMan Pro Lasergene v10.0 (DNA STAR).

3.2. Classification and phylogenetic analysis of soil bacteria based on 16S rRNA gene

16S rRNA gene sequencing and phylogenetic analysis of 126 bacterial clones (Fig. 1A), divides them into 8 groups (Fig. 1B). Group I belongs to Planctomycetes (3%), Group II belongs to Chloroflexi (1%), Group III belongs to Gemmatimonadetes (2%), Group IV belongs to Acidobacteria (10%), Group V belongs to Bacteroidetes (13%), and Group VI belongs to Proteobacteria (67%). Both Group VII (3%) and Group VIII (1%) include bacteria which could not be classified. In Group I, four bacterial clones (uncultured bacterium clone 75, 61, 53, 66) were categorized under phylum Planctomycetes. It shows a close relationship with Group VII of unknown bacterial clones (uncultured bacterium clone 3, 10, 20, 82) and Group II phylum Chloroflexi (uncultured bacterium clone 54) (Fig. 1B). In Group III, two bacterial clones were categorized under the phylum Gemmatimonadetes (uncultured bacterium clone 57 and 79). Group IV characterized as Acidobacteria, contains 13 bacterial clones (uncultured bacterium clone 23, 41, 42, 55, 56, 60, 62, 65, 69, 71, 77, 81, 84). 17 bacterial clones come under Group V Bacteroidetes (uncultured bacterium clone 2, 4, 6–9, 12, 13, 15, 17, 18, 24, 25, 29, 108, 110, 116) and all show similarity with *Flavisolibacter*. Out of 126 bacterial clones, the majority (84 bacterial clones) belong to Group VI Proteobacteria, in which most bacterial clones belong to class Gammaproteobacteria (78.57%), followed by Betaproteobacteria (15.48%) and Alphaproteobacteria (5.95%) (Fig. 1C). In Alphaproteobacteria, uncultured bacterium clone 99 shows similarity with *Bradyrhizobium denitrificans* that belongs to family Bradyrhizobiaceae. Bacterial clone 43 and 44 showed similarity with *Hyphomicrobium* and *Rhodoplanes elegans*, respectively (Family Hyphomicrobiaceae). Bacterium clone 80 and 83 showed similarity with *Sphingomonas* that belong to family Sphingomonadaceae. Ten bacterial clones of Betaproteobacteria showed similarity with *Burkholderia* (Family Burkholderiaceae). Class Gammaproteobacteria is further divided into 5 families. Bacterial clone 88, 90 and 92 show similarity with *Aquicella siphonis* that belongs to family Coxiellaceae; bacterial clone 78 showed similarities with *Acinobacter*

Johnsonii of family Moraxellaceae; bacterial clone 64 and 67 showed similarity with *Steroidbacter* of family Sinobacteraceae; and bacterial clone 58 and 59 showed similarity with *Pseudoxanthomonas* of family Xanthomonadaceae. Fifty one out of 126 bacterial clones belong to family Pseudomonadaceae, and they show similarity with the genus *Pseudomonas*, 45 clones with similarity (99–100%) to *Pseudomonas stutzeri* and 3 clones each to *Pseudomonas balearica* and *Pseudomonas plecoglossicida*. 17 clones with similarity (92–96%) to *Flavisolibacter ginsengisoli*.

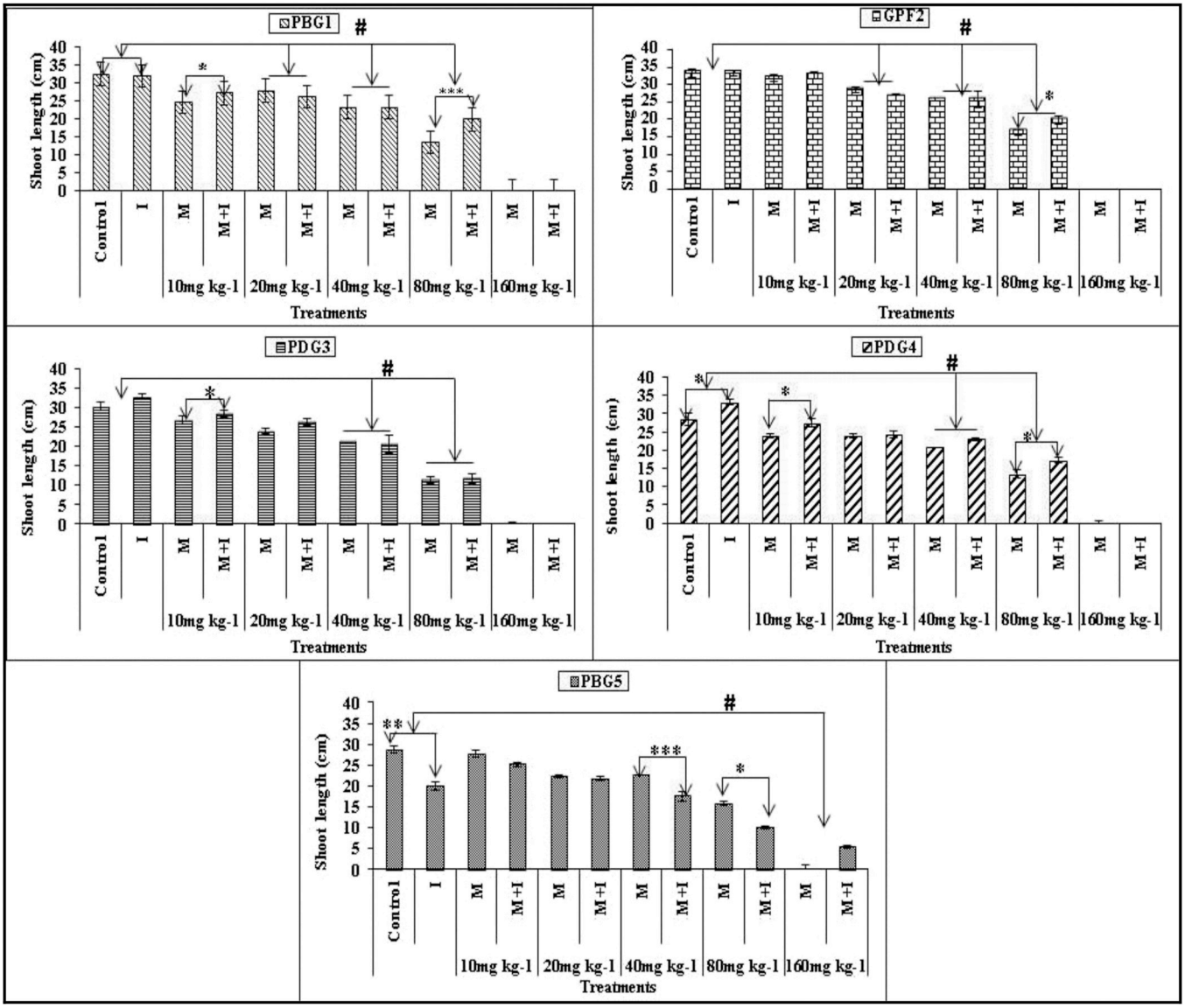

Fig. 2. - Effect of Arsenic on shoot length in chickpea. C-Control, M- Seeds treated with metal, M + I – Seeds treated with metal and bacterial inoculum, I- Seeds treated with only bacterial inoculum. *-represent significance level. *-$p < 0.05$, **-$p < 0.01$ and ***-$p < 0.001$. #-represents significance of different M and M + I treatment with Control and Inoculated control. (2 column).

Based on the analysis above, it can be concluded that the highest proportion (40%) of soil bacteria belong to the genus *Pseudomonas*.

3.3. Response of isolated Pseudomonas spp. against heavy metals and identification of potential plant growth promoting bacteria

Eight *Pseudomonas* isolates (*i.e.*, KM594392, KM594399, KM594393, KM594394, KM594395, KM594397, KM594396, and KM594398) were first tested against toxic heavy metals and metalloids. These isolates showed varying tolerance levels against As^{3+}, As^{5+}, Cr^{2+}, Hg^{2+} and Cd^{2+}. Isolates showed maximum and least tolerance to As^{5+} and Hg^{2+}, respectively (Table 1). However, the pattern of tolerance varied among the isolates. To determine the minimum inhibitory concentrations of isolates, MIC_{100} was determined. MIC_{100} observed for Hg^{2+}, Cr^{2+}, Cd^{2+}, As^{3+} and As^{5+} were 2 mg L^{-1}, 350 mg L^{-1}, 350 mg L^{-1}, 0.5M and 250 mg L^{-1}, respectively. In general, the order of toxicity to these metals was $Hg^{2+} > As^{3+} > Cr^{2+} > Cd^{2+} > As^{5+}$ and among the isolates, KM594394, KM594395 showed highest MIC and MTC values for Cd^{2+}, As^{5+}, and As^{3+}, whereas, KM594393 showed highest tolerance towards Cr^{2+}, Hg^{2+} As^{5+}, and As^{3+} but not for Cd^{2+}. The order of tolerance of the isolates can be said to be KM594393 > KM594394 > KM594399 > KM594395 > KM594397 > KM594398 > KM594396. All the *Pseudomonas* isolates produced Indole Acetic Acid (IAA) and consequently considered as IAA producing bacteria. The production of IAA by *Pseudomonas* isolates was observed only in the presence of L-tryptophan. The range of IAA production by the *Pseudomonas* isolates was 138.12 ± 8.25 μg mL^{-1} to 80.11 ± 9.63 μg mL^{-1} in strains KM594397 and KM594395, respectively (Table 2). Out of the eight isolates, only two isolates (KM594398 and KM594397) were positive for Phosphate solubilisation test with the strain KM594397 recording the highest Phosphate solubilisation index (PSI-2.7) (Table 2).

It was observed that the isolates were more sensitive to Hg^{2+} and least to As^{5+}. All the isolates were IAA producers but only two isolates showed phosphate solubilizing activity. Based on the heavy metal tolerance and PGP characteristics, strain KM594397 *Pseudomonas citronellolis* (PC) was selected for further study on its PGP activity in chickpea under As^{5+} stress.

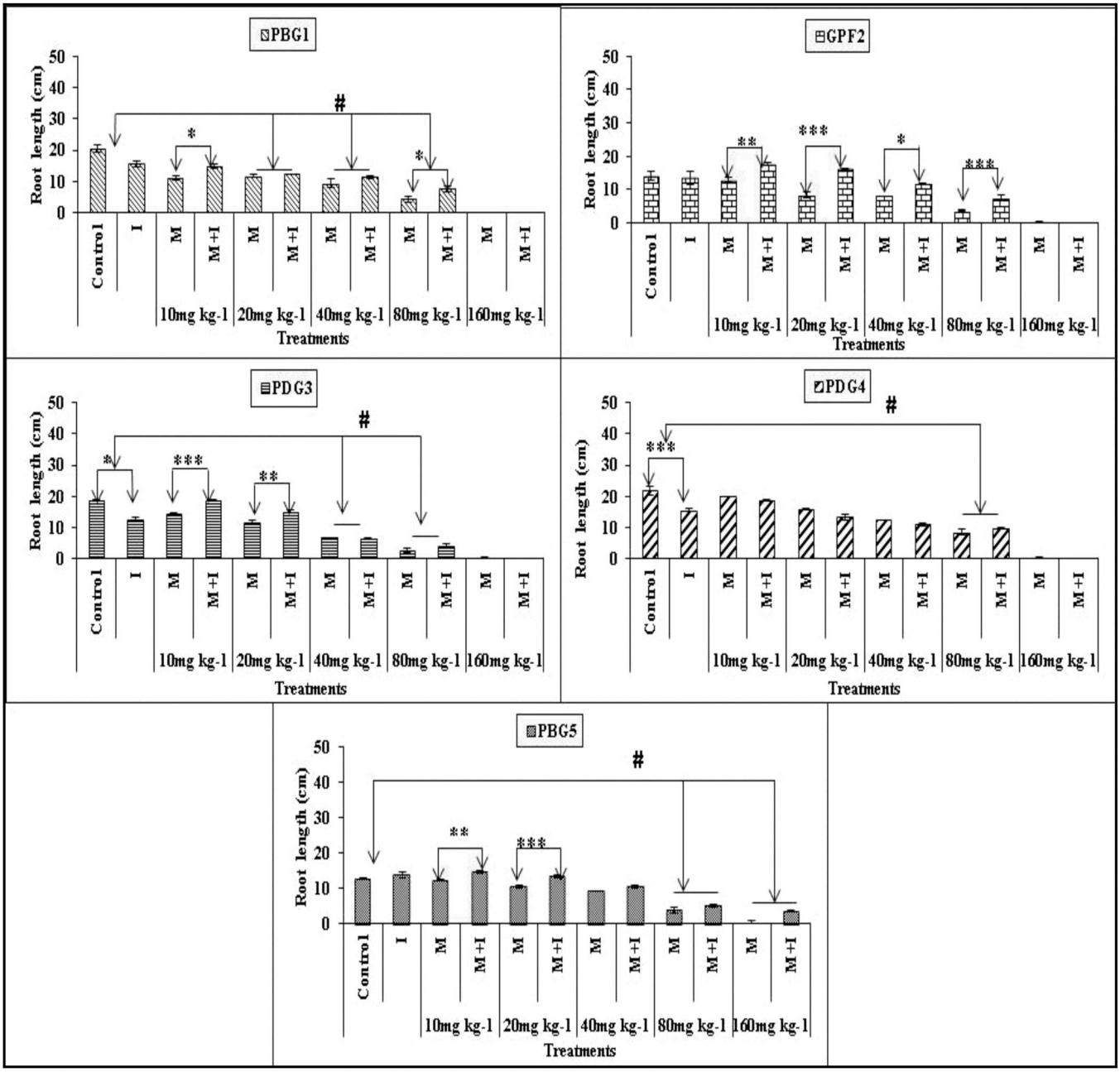

Fig. 3. Effect of Arsenic on root length in chickpea. C-Control, M- Seeds treated with metal, M + I – Seeds treated with metal and bacterial inoculum, I- Seeds treated with only bacterial inoculum. *-represent significance level. *-$p < 0.05$, **-$p < 0.01$ and ***-$p < 0.001$. #-represents significance of different M and M + I treatment with Control and Inoculated control. (2 column).

3.4. Effect of Pseudomonas citronellolis (KM594397) on the growth of chickpea under As^{5+} stress

3.4.1. Effect of PGP Pseudomonas citronellolis on the root and shoot growth under As^{5+} stress

Shoot and root length showed significant variations with respect to metal treatments and also with and without inoculums in different chickpea varieties. Shoot and root length gradually decreased with increase in the As^{5+} concentration. In PBG1 and GPF2, 20 mg kg^{-1}, 40 mg kg^{-1} and 80 mg kg^{-1}, in PDG3 and PDG4, 40 mg kg^{-1} and 80 mg kg^{-1} treatment of As^{5+} showed significant decrease in the shoot length as compared to the normal and inoculated control (Fig. 2), When bacterial inoculum (PC) is used along with As^{5+}, in PBG1 10 mg kg^{-1} and 80 mg kg^{-1}, in GPF2 only 80 mg kg^{-1}, in PDG3 10 mg kg^{-1} and in PDG4 80 mg kg^{-1} As^{5+} treatment showed significant increase in the shoot length as compared to only metal treated plants. PBG 5 showed decrease in the shoot length as compared to normal and inoculated control even in metal + PC inoculated plants. Root length showed similar pattern as observed in shoots. In PBG1, 20 mg kg^{-1}, 40 mg kg^{-1} and 80 mg kg^{-1}, in PDG3, 40 mg kg^{-1} and 80 mg kg^{-1}, in PDG4, 80 mg kg^{-1} and in PBG5, 80 mg kg^{-1} and 160 mg kg^{-1} As^{5+} treatment showed significant decrease in the root length as compared to normal and inoculated control (Fig. 3). In GPF2, there was no significant change in Root length as compared to the control and inoculated control. Inoculation of Bacterial inoculum (PC) along with metal treatments showed increase in the root length as compared to only metal treated plants. In PBG1, 10 mg kg^{-1} and 80 mg kg^{-1}, in GPF2, 10 mg kg^{-1}, 20 mg kg^{-1} 40 mg kg^{-1} and 80 mg kg^{-1} and in PDG3, and

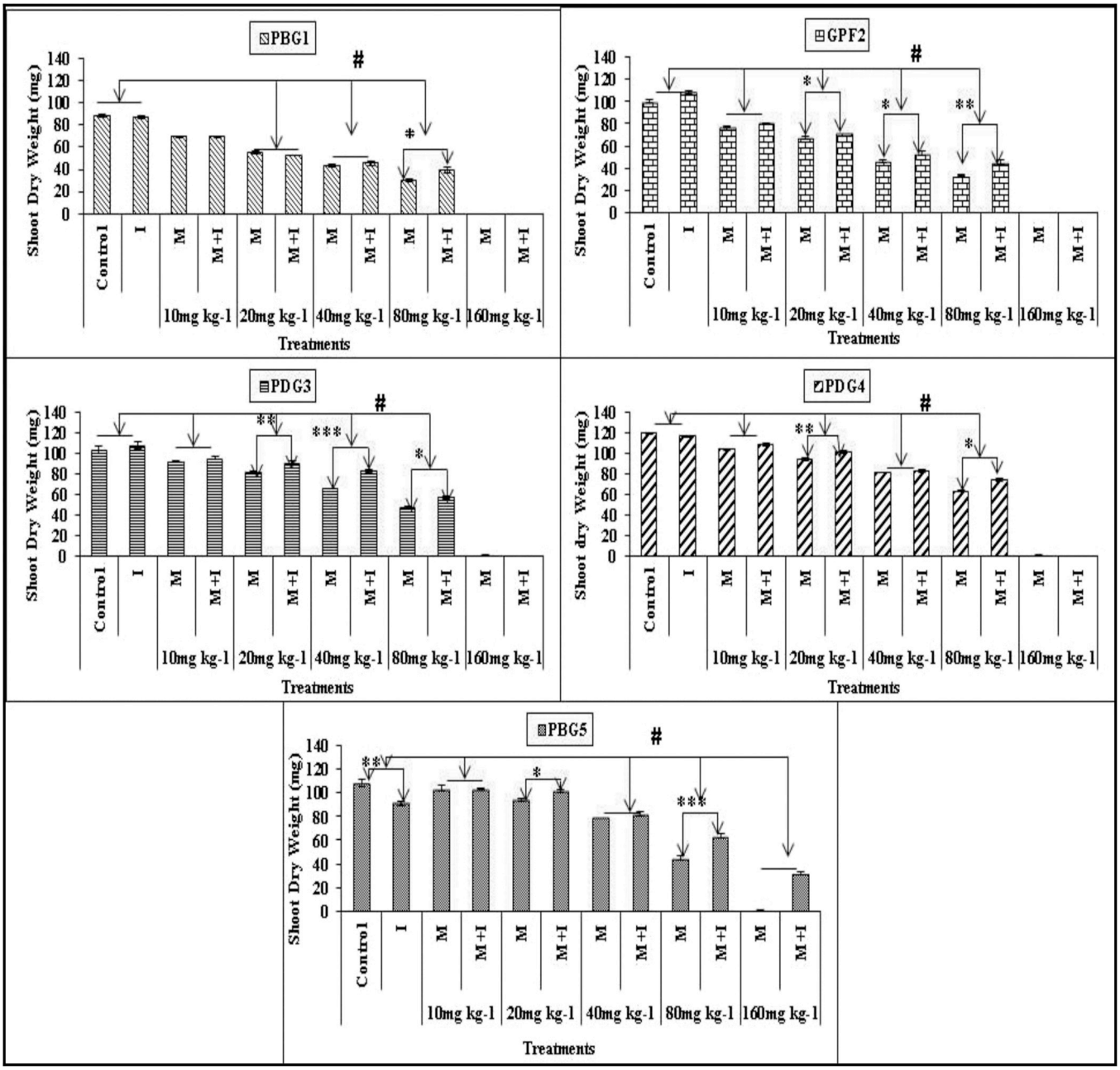

Fig. 4. Effect of Arsenic on shoot dry weight in chickpea. C-Control, M- Seeds treated with metal, M + I – Seeds treated with metal and bacterial inoculum, I- Seeds treated with only bacterial inoculum. *-represent significance level. *-$p < 0.05$, **-$p < 0.01$ and ***-$p < 0.001$. #-represents significance of different M and M + I treatment with Control and Inoculated control. (2 column).

in PBG5, 10 mg kg^{-1} and 20 mg kg^{-1} showed significant increase in root length in metal + inoculum treated plants as compared to only metal treated plants. As^{5+} treatments of 40 mg kg^{-1} and 80 mg kg^{-1} significantly decreased the shoot and root length as compared to the normal control (C) and inoculated control (I) irrespective of the varieties. Higher dose of As^{5+} (160 mg kg^{-1}) was found to be lethal for the chickpea varieties. PBG5 alone showed growth at 160 mg kg^{-1} of As^{5+} when supplemented with the bacterial inoculum. At a concentration of 80 mg kg^{-1}, approximately 50% reduction in the shoot length was observed in all the varieties and 40 mg kg^{-1} of As^{5+} concentration caused ~50% reduction in the root length in all the varieties.

Inoculation of all the varieties with PC resulted in significant increase in the shoot length and root length in metal + inoculum (M + I) treated plants as compared to only metal (M) treated plants at various concentrations of As^{5+}. Root growth was found to be more affected than shoot growth under metal stress and inoculation of PC had more positive effect in root length than in shoot length under metal stress in all the varieties.

3.4.2. *Effect of Pseudomonas citronellolis on root and shoot biomass under As^{5+} stress*

Both shoot and root dry weight showed significant differences among the varieties and treatments. Shoot dry weight (SDW) and root dry weight (RDW) gradually decreased with increase in the As^{5+} concentration. Except PBG1, other four varieties GPF2,PDG3, PDG4 and PBG5 showed significant decrease in the SDW as compared to the normal and inoculated control in all the treatments (Fig. 4), When bacterial inoculum (PC) is used along with As^{5+}, in PBG1, only 80 mg kg^{-1}, in GPF2 and PDG3, 20 mg kg^{-1}, 40 mg kg^{-1} and 80 mg kg^{-1} and in PDG4 and PBG5, 20 mg kg^{-1} and 80 mg kg^{-1} As^{5+}

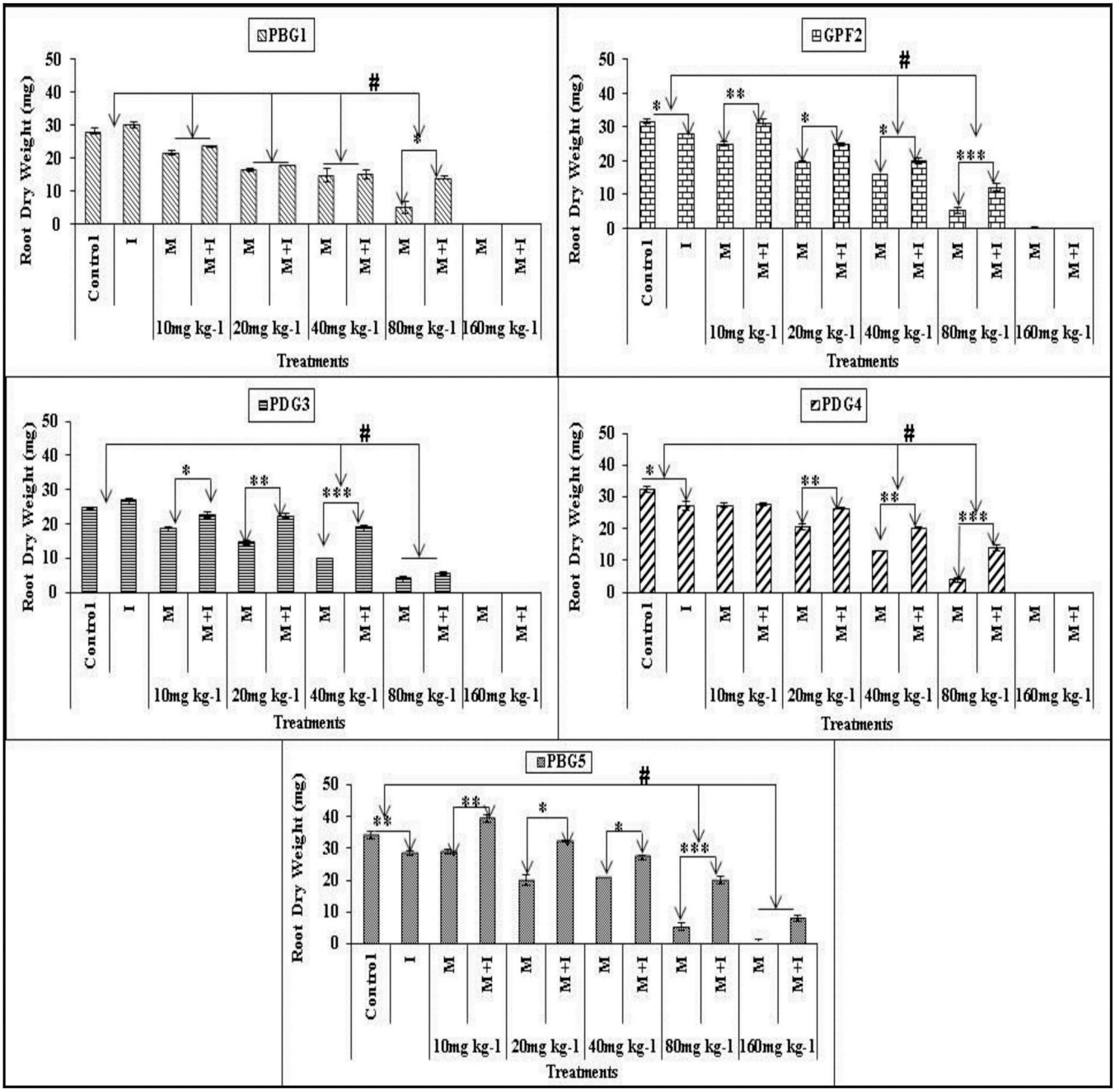

Fig. 5. Effect of Arsenic on root dry weight in chickpea. C-Control, M- Seeds treated with metal, M + I – Seeds treated with metal and bacterial inoculum, I- Seeds treated with only bacterial inoculum. *-represent significance level. *-$p < 0.05$, **-$p < 0.01$ and ***-$p < 0.001$. #-represents significance of different M and M + I treatment with Control and Inoculated control. (2 column).

treatment showed significant increase in the SDW as compared to only metal treated plants. RDW showed similar pattern as with SDW. In PBG1, all the treatments, in GPF2, PDG3 and PDG4 40 mg kg^{-1} and 80 mg kg^{-1}, and in PBG5, 80 mg kg^{-1} and 160 mg kg^{-1} $As5^{+}$ treatments showed significant decrease in the RDW as compared to normal and inoculated control (Fig. 5). Inoculation of Bacterial inoculum along with metal treatments showed increase in the RDW as compared to only metal treated plants. In PBG1, 80 mg kg^{-1}, in GPF2 and PBG5, 10 mg kg^{-1}, 20 mg kg^{-1}, 40 mg kg^{-1} and 80 mg kg^{-1}, in PDG3, 10 mg kg^{-1}, 20 mg kg^{-1} and 40 mg kg^{-1} and in PDG4, 20 mg kg^{-1}, 40 mg kg^{-1} and 80 mg kg^{-1} showed significant increase in RDW in metal + inoculum treated plants as compared to only metal treated plants. As^{5+} treatment with 40 mg kg^{-1} and 80 mg kg^{-1} significantly reduced the SDW and RDW as compared to the normal control (C) and inoculated control (I) in all five varieties. 80 mg kg^{-1} As^{5+} caused ~50% reduction in the SDW in all the varieties except in PBG1 and GPF2 whereas 40 mg kg^{-1} As^{5+} treatment showed ~50% reduction in the SDW. In case of RDW, 40 mg kg^{-1} As^{5+} treatment caused ~50% reduction in almost all the varieties.

Plants treated with PC along with metal (M + I) have shown increased SDW and RDW as compared to only metal (M) treated plants. At 80 mg kg^{-1}, SDW and RDW increased significantly in PC treated plants with metal treatment (M + I) as compared to only metal treated (M) plants. Overall, bacterial inoculated plants showed better SDW and RDW as compared to only metal treated plants in all the five varieties.

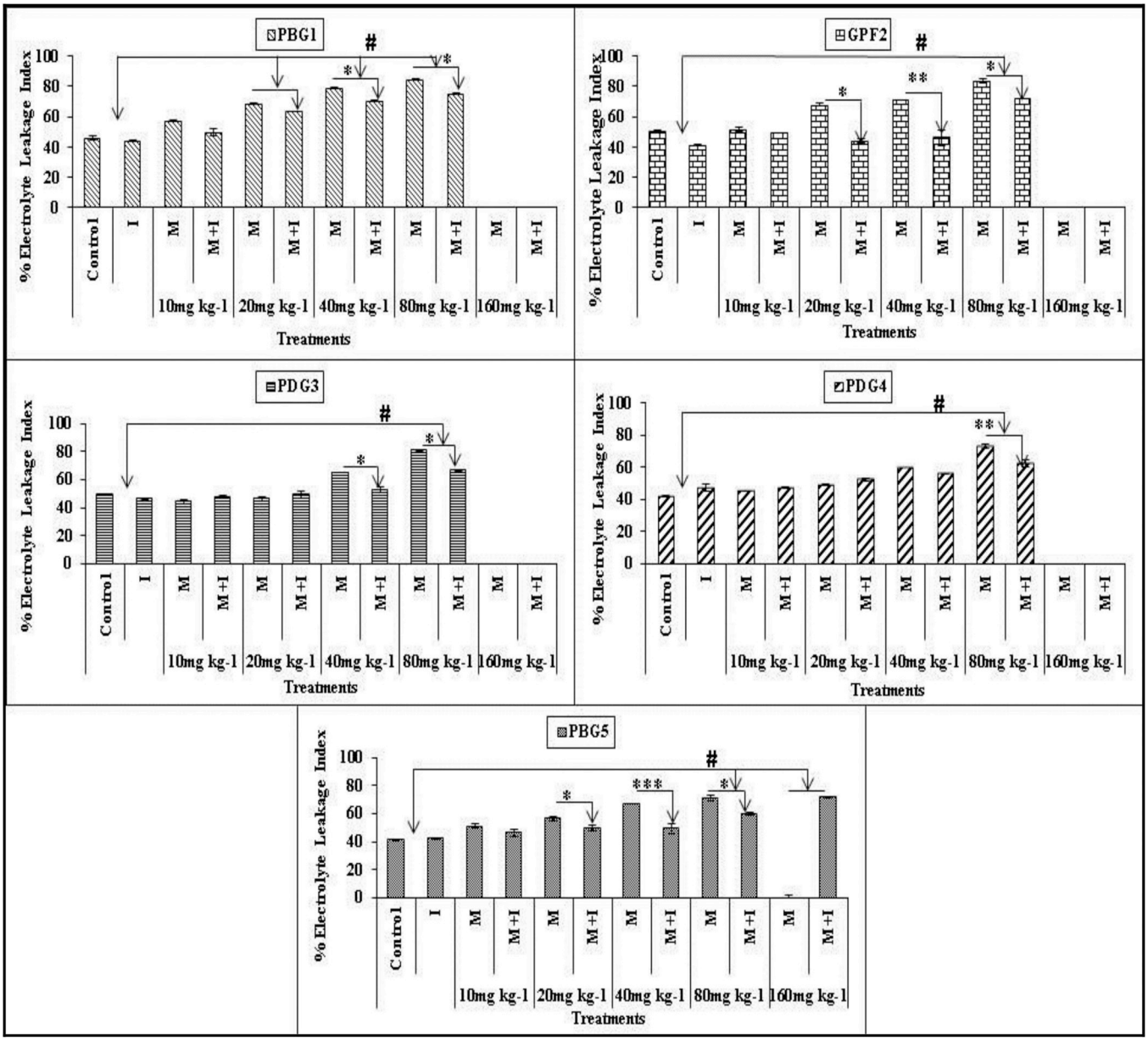

Fig. 6. Effect of Arsenic on Electrolyte leakage index in chickpea. C-Control, M- Seeds treated with metal, M + I – Seeds treated with metal and bacterial inoculum, I-Seeds treated with only bacterial inoculum. *-represent significance level. *-$p < 0.05$, **-$p < 0.01$ and ***-$p < 0.001$. #-represents significance of different M and M + I treatment with Control and Inoculated control. (2 column).

3.5. Effect of Pseudomonas citronellolis on membrane integrity of chickpea under As^{5+} stress

3.5.1. Effect of Pseudomonas citronellolis on electrolyte leakage index (ELI) under As^{5+} stress

Electrolyte leakage index is a measure of membrane integrity of the plant. ELI gradually increased with increase in the As^{5+} concentration. All the varieties showed significant increase in the ELI as compared to the normal and inoculated control in all the treatments (Fig. 6), In PBG1, 20 mg kg^{-1}, 40 mg $kg^{-1}$and 80 mg kg^{-1}, in GPF2, PDG3 and PDG4, 80 mg kg^{-1} and in PBG5 80 mg kg^{-1} and 160 mg kg^{-1} As^{5+} treatment showed significant increase in the ELI as compared to the control (C) and inoculated control (I). When seeds were inoculated with bacterial inoculum along with As^{5+}, in PBG1 and PDG3 40 mg kg^{-1} and 80 mg kg^{-1}, in GPF2 and PBG5, 20 mg kg^{-1}, 40 mg kg^{-1} and 80 mg kg^{-1} and in PDG4 only 80 mg kg^{-1} $As5^{+}$ treatment showed significant decrease in the ELI as compared to only metal treated plants. As^{5+} treatment of 40 mg kg^{-1} and 80 mg kg^{-1} showed significant increase in the ELI as compared to the normal control plants and inoculated control (I) of all varieties. The ELI was found to increase by ≥ 50% in all varieties at As^{5+} concentrations 40 mg $kg^{-1}$and 80 mg kg^{-1}.

Metal treated (M + I) plants inoculated with bacteria (PC) showed less ELI as compared to only metal (M) treated plants. At 40 mg kg^{-1} and 80 mg kg^{-1} As^{5+} treatment, all the varieties showed significant decrease in the ELI. PBG1 showed highest ELI as compared to the other varieties whereas, PBG5 showed lowest ELI among all the varieties.

3.5.2. Effect of Pseudomonas citronellolis on malondialdehyde (MDA) content under As^{5+} stress

Malondialdehyde (MDA) content is a measure of membrane lipid peroxidation. MDA content increased gradually with increase in the As^{5+} concentration. All the varieties showed significant increase in the MDA content as compared to the normal and inoculated control in all

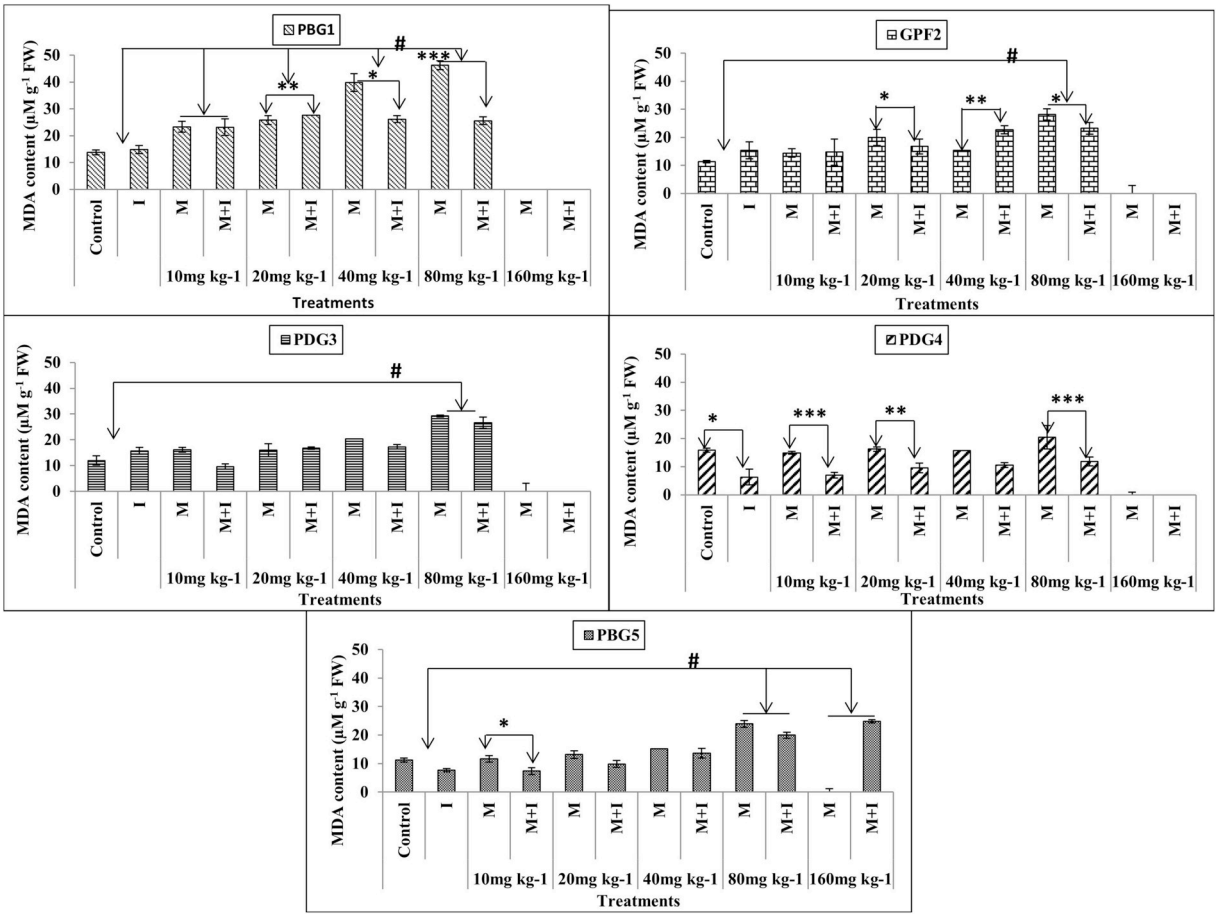

Fig. 7. Effect of Arsenic on MDA content in chickpea. C-Control, M- Seeds treated with metal, M + I – Seeds treated with metal and bacterial inoculum, I- Seeds treated with only bacterial inoculum. *-represent significance level. *-$p < 0.05$, **-$p < 0.01$ and ***-$p < 0.001$. #-represents significance of different M and M + I treatment with Control and Inoculated control. (2 column).

the treatments (Fig. 7), In PBG1, 10 mg kg^{-1}, 20 mg kg^{-1}, 40 mg kg^{-1} and 80 mg kg^{-1}, in GPF2 and PDG3 80 mg kg^{-1} and in PBG5 80 mg kg^{-1} and 160 mg kg^{-1} As^{5+} treatment showed significant increase in the MDA content as compared to normal control (C) and inoculated control (I). When seeds were inoculated with bacterial inoculum (PC) along with As^{5+}, in PBG1 and GPF2, 20 mg kg^{-1}, 40 mg kg^{-1} and 80 mg kg^{-1}, in PDG4 10 mg kg^{-1}, 20 mg kg^{-1}, and 80 mg kg^{-1} and in PBG 5 only 10 mg kg^{-1} As^{5+} treatment showed significant decrease in the MDA content as compared to only metal treated plants. As^{5+} treatment (40 mg kg^{-1} and 80 mg kg^{-1}) showed significant increase in the MDA content as compared to the control and inoculated control in all varieties. The MDA content was found to be wide-ranging among varieties, PBG1 showed highest MDA content as compared to the other varieties. Even at 10 mg kg^{-1} As^{5+} treatments it showed 69% increase in the MDA content as compared to the control.

Inoculation of PC has decreased the MDA content in metal + inoculum (M + I) treated plants as compared to only metal (M) treated plants in all the varieties. These results indicate that the plants inoculated with bacteria showed less membrane damage as compared to only metal treated plants.

3.6. *Effect of Pseudomonas citronellolis on chlorophyll content under As^{5+} stress*

Chlorophyll A content in chickpea leaves decreased gradually with increase in the As^{5+} concentration. In PBG1, 20 mg kg^{-1} and 40 mg kg^{-1}, in PDG3, 40 mg kg^{-1} and 80 mg kg^{-1} and in PBG5 20 mg kg^{-1}, 40 mg kg^{-1}, 60 mg kg^{-1}, 80 mg kg^{-1} and 160 mg kg^{-1} of As^{5+} treated plants showed significant decrease in the Chlorophyll content as compared to the normal control (C) and inoculated control (I) (Table 3). Inoculation of PC increased Chlorophyll A content in M + I treated plants than metal treated plants. In GPF2, 80 mg kg^{-1}, in PDG4 10 mg kg^{-1} and in PBG5 10 mg kg^{-1}, 20 mg kg^{-1}, 40 mg kg^{-1}, and 80 mg kg^{-1} showed significant higher Chlorophyll A content in M + I treated plants as compared to metal treated plants. In case of Chlorophyll B, increase in the As^{5+} concentration resulted in the decrease in the Chlorophyll B content (Table 3**).** In PBG1, 20 mg kg^{-1}, 40 mg kg^{-1}, 80 mg kg^{-1}, in PDG3, 80 mg kg^{-1} and in PBG5, 80 mg kg^{-1} and 160 mg kg^{-1} As^{5+} treatment showed significant decrease in the Chlorophyll content as compared to the normal control (C) and inoculated control (I). PC inoculation showed higher chlorophyll B content in M + I treated plants than metal treated plants. In GPF2, 80 mg kg^{-1}, in PDG4, 10 mg kg^{-1}, 20 mg kg^{-1}, and 40 mg kg^{-1} and in PBG5, 10 mg kg^{-1}, 20 mg kg^{-1}, 40 mg kg^{-1}, 80 mg kg^{-1}.

Overall, metal + inoculum (M + I) treated plants showed significant increase in chlorophyll content than only metal treated plants and PBG 5 variety showed highest chlorophyll content among the varieties.

Both Chlorophyll A and Chlorophyll B showed significant increase in the metal + Inoculum (M + I) treated plants as compared to the only metal (M) treated plants in PBG5. Chlorophyll B showed the same trend in PDG4 variety. In PBG5, both Chlorophyll A and B showed significant increase in inoculated control (I) than normal control (C). PDG4 and GPF2 did not show significant variation in chlorophyll content as compared to normal (C) and inoculated control (I). The tolerant variety PBG5 showed positive interaction with the PGPB and showed higher

Table 3
Chlorophyll content and Chlorophyll A/B ratio in Chickpea under As^{5+} stress.

Variety	Treatments		Chlorophyll A μg mg^{-1}	Chlorophyll B μg mg^{-1}	Chlorophyll A/B
PBG1	**Control**	**C**	2.98 ± 0.27	5.41 ± 0.49	0.55 ± 0.00
		I	3.02 ± 0.64	5.49 ± 1.17	0.55 ± 0.00
	10 mg kg^{-1}	**M**	2.03 ± 0.74	4.18 ± 1.10	0.48 ± 0.02
		M + I	2.30 ± 0.60	4.31 ± 0.24	0.53 ± 0.07
	20 mg kg^{-1}	**M**	1.96 ± 0.69#	3.56 ± 1.27#	0.55 ± 0.00
		M + I	2.10 ± .56#	3.70 ± 1.36#	0.57 ± 0.03
	40 mg kg^{-1}	**M**	1.68 ± .93#	3.18 ± 0.34#	0.53 ± 0.12
		M + I	1.74 ± .18#	3.82 ± 1.01#	0.46 ± 0.06
	80 mg kg^{-1}	**M**	2.37 ± .13	2.92 ± 0.66#	0.81 ± 0.06
		M + I	1.60 ± 0.37	3.07 ± 1.66#	0.53 ± 0.07***
	160 mg kg^{-1}	**M**			
		M + I			
GPF2	**Control**	**C**	3.09 ± 1.33	4.88 ± 0.46	0.63 ± 0.09
		I	2.67 ± .04	4.86 ± 0.07	0.55 ± 0.00
	10 mg kg^{-1}	**M**	2.74 ± 1.09	4.98 ± 1.98	0.55 ± 0.00
		M + I	2.74 ± 0.81	5.61 ± 2.40	0.50 ± 0.13
	20 mg kg^{-1}	**M**	2.77 ± 0.38	4.72 ± 0.95	0.58 ± 0.03
		M + I	2.72 ± 0.90	5.03 ± 0.70	0.54 ± 0.09
	40 mg kg^{-1}	**M**	2.68 ± 0.25	4.51 ± 2.32	0.61 ± 0.16
		M + I	2.59 ± 0.51	4.99 ± 1.47	0.52 ± 0.08
	80 mg kg^{-1}	**M**	1.57 ± 0.074	2.85 ± .014	0.55 ± 0.00
		M + I	2.48 ± 1.28**	4.94 ± 1.63***	0.51 ± 0.16
	160 mg kg^{-1}	**M**			
		M + I			
PDG3	**Control**	**C**	2.87 ± 0.45	5.23 ± 0.82	0.55 ± 0.00
		I	3.22 ± 0.31	5.84 ± .57	0.55 ± 0.00
	10 mg kg^{-1}	**M**	2.61 ± 0.41	4.52 ± .98	0.58 ± 0.03
		M + I	3.10 ± 0.65	5.63 ± 1.18	0.55 ± 0.00
	20 mg kg^{-1}	**M**	2.48 ± 0.54	3.89 ± 1.99	0.64 ± 0.08
		M + I	2.38 ± 0.58	4.75 ± 0.75	0.50 ± 0.07
	40 mg kg^{-1}	**M**	1.94 ± 0.56#	3.37 ± 0.36	0.57 ± 0.08
		M + I	2.14 ± 1.01#	4.34 ± 1.05	0.50 ± 0.15
	80 mg kg^{-1}	**M**	1.86 ± 0.198#	3.53 ± 1.02#	0.53 ± 0.07
		M + I	2.07 ± 0.23	3.77 ± 0.43#	0.55 ± 0.00
	160 mg kg^{-1}	**M**			
		M + I			
PDG4	**Control**	**C**	2.64 ± 1.38	4.78 ± 2.51	0.55 ± 0.00
		I	2.72 ± 0.46	4.49 ± 0.47	0.60 ± 0.06
	10 mg kg^{-1}	**M**	2.25 ± 0.46	4.09 ± 0.84	0.55 ± 0.00
		M + I	3.42 ± 0.84**	5.58 ± 1.86[a]	0.61 ± 0.08
	20 mg kg^{-1}	**M**	2.47 ± 0.25	3.94 ± 0.90	0.62 ± 0.03
		M + I	3.07 ± 1.02	6.21 ± 1.53***	0.49 ± 0.06
	40 mg kg^{-1}	**M**	2.17 ± 0.49	3.18 ± 0.59	0.68 ± 0.02
		M + I	1.83 ± 0.34	4.93 ± 0.84**	0.37 ± 0.05[a]
	80 mg kg^{-1}	**M**	1.67 ± .75	3.03 ± 1.37	0.55 ± 0.00
		M + I	1.75 ± 0.32	3.32 ± 0.61	0.52 ± 0.03
	160 mg kg^{-1}	**M**			
		M + I			
PBG5	**Control**	**C**	3.65 ± 0.09	4.75 ± 1.86	0.59 ± 0.08
		I	4.43 ± 1.19***	6.21 ± 2.17***	0.55 ± 0.00
	10 mg kg^{-1}	**M**	2.42 ± 0.91	4.34 ± 1.66	0.55 ± 0.00
		M + I	3.76 ± 1.07***	4.09 ± 1.94***	0.55 ± 0.00
	20 mg kg^{-1}	**M**	2.19 ± 0.74	3.77 ± 0.42	0.75 ± 0.07
		M + I	3.40 ± 1.02***	3.94 ± 0.18***	0.51 ± 0.06***
	40 mg kg^{-1}	**M**	1.60 ± 0.23	5.84 ± 0.58	0.59 ± 0.07
		M + I	2.85 ± 1.80***	3.18 ± 1.28***	0.55 ± 0.00
	80 mg kg^{-1}	**M**	1.46 ± 0.24	4.78 ± 0.45#	0.55 ± 0.00
		M + I	2.26 ± [a]0.33	3.03 ± 0.61[a]#	0.55 ± 0.00
	160 mg kg^{-1}	**M**			
		M + I	3.77 ± 0.31#	5.58 ± 1.35#	0.38 ± 0.08#

[a] C-Control, M- Seeds treated with metal, M + I – Seeds treated with metal and bacterial inoculum, I- Seeds treated with only bacterial inoculum. *-represent significance level. *-$p < 0.05$, **-$p < 0.01$ and ***-$p < 0.001$. #-represents significance of different M and M + I treatment with Control and Inoculated control.

Chlorophyll content in metal + inoculated (M + I) and only inoculated (I) plants. Chlorophyll A/B ratio was also calculated. At higher concentration of As^{5+}, Chlorophyll A/B ratio increased as compared to control (C) and inoculated control (I). Inoculation of PC resulted in decreased Chlorophyll A/B ratio in M + I treated plants than metal (M) treated plants. In PBG1, at 80 mg kg^{-1}, in PDG4, 40 mg kg^{-1} and in PBG5, at 20 mg kg^{-1}, metal + inoculum (M + I) treatment showed significant decrease in the Chlorophyll A/B ratio than the corresponding only metal (M) treated plants.

4. Discussion

Heavy metal contamination of soil and water has become a major concern for human and plant health. It severely affects plant growth and development and reduces agriculture production, which is alarming situation for growing population. Metal tolerant soil bacteria with plant growth promoting properties can be an option for plant growth promotion in heavy metal contaminated sites. Microbial diversity analysis using16S rRNA gene sequences from soil showed that

the microbes belong to eight different categories of phylogenetic groups, *i.e.*, Planctomycetes, Chloroflexi, Gemmatimonadetes, Acidobacteria, Bacteroidetes, Proteobacteria and two unknown groups. Out of these eight phylogenetic groups, Proteobacteria is abundant (~67%), followed by Bacteroidetes (~13%) and Acidobacteria (~10%). In one of a previous study, 16S rRNA gene analysis of soil showed presence of Proteobacteria, Acidobacteria, Planctomycetes, Chloroflexi, Bacteroidetes, Verrucomicrobia, Nitrospirae, Actinobacteria, out of which Proteobacteria was the most predominant (Zhou et al., 2009). Later, Yin et al. (2015) reported that under heavily contaminated environment, there is a decrease in Proteobacteria and Actinobacteria and increase in Firmicutes, Chloroflexi and Crenarchaeota population. In present study, Gammaproteobacteria is abundant followed by Beta and Alphaproteobacteria. Deltaproteobacteria was not found. At the genus level, *Pseudomonas* (40.48%) is predominant followed by *Flavisolibacter* (13.49%) and Burkholderia spp. (7.94%). The predominance of *Pseudomonas* implies that it is the most resistant to the heavy metal contamination. Most of the previous studies have shown *Pseudomonas* resistance towards high levels of heavy metal content, and they might have the potential of denitrification, nitrogen fixation, biosorption, degradation, accumulation, bioremediation and reduction of heavy metals and metalloids (Lalucat et al., 2006).

Eight strains of *Pseudomonas* having tolerance to essential metal elements such as Cu^{2+}, Zn^{2+} and Fe^{3+} were isolated (Adhikary et al., 2019). The eight isolated *Pseudomonas* strains showed resistance to multiple heavy metals along with plant growth promoting activity. In the present study, the MIC and MTC for Cr^{2+}, Cd^{2+}, As^{5+} was found to be higher as compared to previous studies conducted by Singh et al. (2010). High metal resistance in bacteria is an important factor to be considered for plant growth in heavy metal contaminated sites. In fact, multiple metal resistant bacteria are promising candidates for plant growth promotion under heavy metal stress because many sites are co-contaminated with multiple heavy metals. In previous studies, bacterial isolates that displayed resistance to one metal also showed resistance towards several other metals (Malik and Aleem, 2011).

Production of plant hormone and Phosphate solubilisation by heavy metal resistant bacteria is an additional characteristic which generally promote plant growth under metal stress. Based on the relatively high Phosphate solubilisation, IAA production and multiple heavy metal resistance, strain KM594397 was selected for Arsenic (As^{5+}) toxicity and plant growth experiment. Plant–bacterium interaction is a crucial factor for plant growth promotion by the PGPB. IAA production and Phosphate solubilisation by the PGPB provide the plant with necessary nutrient phosphate and auxin for plant growth and development. Application of KM594397 increased the chickpea growth and dry matter under metal stress as compared to only metal treated plants. It was previously reported that phosphate solubilizing bacteria stimulate plant growth through enhancement of P-nutrition (Rajkumar and Freitas, 2008; Whitelaw, 1999) and also by increasing the N, P, K, Fe uptake (Biswas et al., 2000). Phosphorus availability in soil can increase the plant growth by increasing the efficiency of biological nitrogen fixation. Co-inoculation with PGPB and *Rhizobium* spp. was found to increase root and shoot biomass and grain yield in chickpea plants (Gull et al., 2004). Recently, Cu-resistant *Pseudomonas* endowed with plant growth promoting traits was found to mobilize Cu and improved maize and sunflower health under Cu stress (Li and Ramakrishna, 2011). In one of the study, heavy metal resistant plant growth promoting *Pseudomonas* improved *Ricinus communis* growth in Ni, Cu and Zn contaminated soil (Rajkumar and Freitas, 2008). In another study, Wani and Khan (2010) demonstrated that chromium reducing PGPB *Bacillus* PSB10 reduces the chromium uptake and improves growth, nodulation, chlorophyll, leghaemoglobin, seed yield and grain protein in chickpea. Srivastava and Singh (2014) and Tripathi et al. (2017), demonstrated that Arsenic tolerant *Trichoderma* sp. and *Acinetobacter* sp. ameliorate the effect of Arsenic on the growth of chickpea. In another study by Das and Sarkar (2018) showed Arsenic resistant *Acinetobacter lwoffii* enhances *Vigna radiata* growth under Arsenic stress. In our study application of KM594397 was also found to reduce membrane damage and increase in the photosynthesis pigment in chickpea under Arsenic stress as evident by the reduction in MDA content, ELI, Chlorophyll A/B ratio and increase in Chlorophyll A and B in the metal + inoculum (M + I) treated plants as compared to only metal treated plants. Afzal et al. (2017) reported that PGPB *Pseudomonas putida* Bj05, *Pseudomonas fluorescens* Ps14, and *Enterobacter* spp. Le14, So02 and Bo03 increase the Chlorophyll content in *Panicum virgatum* L. under Cd^{2+} stress. Inoculation of Ni-resistant *Psychrobacter* spp. Strain SRS8 improves Chlorophyll content in *Ricinus communis* and *Helianthus annuus* under Ni-stress (Ma et al., 2010). In one of our recent study it is reported that PGPB, *Pseudomonas* and *Serratia* enhance macro and micronutrient level (N, P, Ca, Mn, Ni, Cu, Zn) and influence growth in chickpea varieties grown in local soil (Dogra et al., 2019). Hence, it is evident that bacteria surviving in metal contaminated soil can have multiple metal resistances. Isolated *Pseudomonas* spp. including KM594397 possesses multiple metal tolerance and plant growth promoting traits such as P-solubilisation and phytohormones production. The PGPB is free-living and its mutualistic association with the plant can enhance the capability of the plant to different external stress causing factors and growth parameters (Afzal et al., 2017; Dogra et al., 2019; Li and Ramakrishna, 2011).

5. Conclusion

Heavy metal pollution in soil and water is increasing day by day due to the rapid increase in industrial development and human per capita usage of metals. Hence, it is necessary to develop novel strategies to improve plant growth in metal contaminated sites for remediation followed by enhanced agriculture production. Heavy metal resistant bacterial species with PGP like activities were explored by researchers (Pandey et al., 2013; Sheng et al., 2008) for betterment of plant health and production. The present study demonstrated that *Pseudomonas* residing in the heavy metal contaminated soil possessed heavy multimetal (As, Cd, Cr Hg) resistance along with potential plant growth promoting (PGP) characteristics. Strain KM594397 (*P. citronellolis*) performed best for PGPB traits and also showed plant growth promotion in chickpea under As^{5+} (10–160 mg kg^{-1}) stress. Among the varieties, PBG1 was found to be the most sensitive to the As5+ stress and PBG5, the most tolerant variety. The metal resistant *Pseudomonas* spp. isolated and characterized in the present study can be a potential candidate for developing green technology for plant growth and increased agricultural production under heavy metal stress in heavy metal contaminated soil.

Contribution

AA has mainly executed the work; RK has done bacterial community analysis, RP has done the PGP characterisation of the isolate, PB has done the sequencing, RW has co-supervised and SK has conceptualized, guided and prepared the final manuscript. Authors are thankful to Central University of Punjab, Bathinda for providing necessary infrastructure.

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

µL: Microlitre
^0C: Degree centigrade
ANOVA: Analysis of Variance
As^{3+}: Arsenite
As^{5+}: Arsenate
$Cd2^+$: Cadmium
CFU: Colony Forming Unit
Cm: Centimetre
Cr^{2+}: Chromium
Cu^{2+}: Copper
dNTP: Deoxyribonucleoside triphosphate
ELI: Electrolyte Leakage Index
Fe^{3+}: Iron
H: hour
Hg^{2+}: Mercury
IAA: Indole Acetic Acid
M: Metal
M + I: Metal + Inoculum
MDA: Malondialdehyde
$MgCl_2$: Magnesium chloride
MIC: Minimum Inhibitory Concentration
Min: Minutes
mM L^{-1}: Millimol per litre
m: Meter
MTC: Maximum Tolerance Capacity (MTC)
Ng: Nanogram
Ni^{2+}: Nickel
Pb^{2+}: Lead
PC: Pseudomonas citronellolis
PCR: Polymerase Chain Reaction
PGPB: Plant Growth Promoting Bacteria
PSI: Phosphate Solubilisation Index
PVK: Pikovskaya Agar Medium
RDW: Root dry weight
RPM: Revolutions per minutes
rRNA: Ribosomal Ribonucleic Acid
SDW: Shoot dry weight
TCP: Tri-calcium Phosphate
U: Unit