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RESPONSE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) IN RELATION TO ELEVATED TEMPERATURE CONDITIONS IN GROUNDNUT (*ARACHIS HYPOGAEA* L.)

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ABSTRACT

The increase in carbon dioxide and temperature under climate change scenario has been shown to increase plant growth as well as the allocation of nutrients to the below ground ecosystem. This allocation may result in alteration of plant-microbe interaction, specifically Plant Growth Promoting Rhizobacterial (PGPR) population which is dependent on rhizodepostion. This study investigated the impact of elevated temperature on some PGPR populations in rhizosphere of groundnut variety B-95. Rhizosphere samples were collected at different phenological stages. Various strains of *Pseudomonas* spp., *Enterobacter* spp., *Azotobacter* spp. and *Acetobacter* spp. were enumerated, isolated and tested for several PGP traits such as Indole Acetic Acid (IAA), siderophore, cyanide production etc. Results showed consistent statistically significant differences in the numbers of bacteria between ambient and above ambient temperature treatments. Total 62 isolates represented 32.26% *Pseudomonas*, 27.42% *Enterobacter* 20.97% *Azotobacter* and 19.35% *Acetobacter* population. 74.19% of the total isolates were able to produce IAA whereas 69.35% of the total isolates were positive for NH_3 production. *Pseudomonas* spp. were strongest exhibiters of protease activity (75%). 25 and 32 strains could display antagonism for *Aspergillus niger* and *Fusarium moniliforme* respectively. No loss or gain of PGP traits as a result of elevated temperature could be observed.

KEY WORDS
PGPR
Groundnut
Elevated temperature
Pseudomonas, *Enterobacter*

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INTRODUCTION

Groundnut is considered as an important leguminous as well as oilseed crop worldwide and it requires a long and warm growing season with abundance of sunshine (Fletcher *et al.*, 1992). According to IPCC AR5 (2013) global climate change with a warming of 0.85°C over the last century is expected to have radical impacts on different ecosystems and therefore groundnut, because of its thermo-sensitive nature may appear as a vulnerable crop plant in the present climate change scenario.

Plant growth promoting rhizobacteria are a heterogeneous group of free-living, soil borne bacteria, which are known to enhance the growth of the plant directly by providing nutrients to plants or indirectly by reducing the damage from soil-borne plant pathogens (Kloepper *et al.*, 1980). Rhizospheric soil of groundnut has been shown to host a number of Plant Growth Promoting Rhizobacteria. These beneficial plant associated bacteria play a key role in supporting and/or increasing plant health and growth in both managed and natural ecosystems (Compant *et al.*, 2010). They have been known to stimulate and enhance plant growth directly as they can improve and mobilize the supply of nutrients, such as nitrogen (Dobbelaere *et al.*, 2003; Ahemad, 2012) and phosphorous (Rashid *et al.*, 2004) and make them available for plant uptake or by production of phytohormones and growth regulators (Choong *et al.*, 2003; Stepanova *et al.*, 2008; Ahemad and Malik, 2011) as well as indirectly by promoting plant development by the suppression of pathogens by resisting, inhibiting or other mechanisms of antibiosis (Milner *et al.*, 1996; Hayat *et al.*, 2010; Rajkumar *et al.*, 2010), iron sequestration by siderophores (Bar-ness *et al.*, 1992), HCN (Keremer and Souissi, 2001), cell wall degrading enzymes like chitinase, protease and cellulase etc. (Ajit *et al.*, 2006), and improving soil structure by ameliorating soil and sequestering toxic heavy metal species from polluted soils and degrading xenobiotic compounds (like pesticides) and stress alleviation such as high salinity (Moreira *et al.*, 2014; Ali *et al.*, 2014). In the past few years, there has been an increase in the number of PGPR that have been identified mainly because the role of the rhizosphere as an ecosystem has gained importance in the functioning of the biosphere. Various species of bacteria like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to enhance the plant growth (Okon and Labandera-Gonzalez, 1994; Glick, 1995; Joseph *et al.*, 2007).

Although much is known about these PGPR in groundnut rhizosphere there is very little information regarding the effect of temperature stress on the various PGP traits in different PGPR in relation to the present and future climate change scenario. Moreover, elevated temperature conditions are also likely to modify the ecosystem functioning including the role of various PGPR that are directly

dependent on rhizodepostion. Therefore, the present study was undertaken to study the impact of temperature on PGPR which may play a pivotal role in plant-microbe interaction.

MATERIALS AND METHODS

Field design and sampling

The field experiments were conducted at Research farm of Indian Agricultural Research Institute (IARI), New Delhi. Groundnut (B-95) was sown in four tunnels with different temperature treatments viz. ambient temperature condition from germination to maturity (T1), elevated temperature condition (+2-3ºC) from germination to flowering (T2), elevated temperature condition (+2-3ºC) from flowering to maturity (T3) and elevated temperature condition (+2-3ºC) from germination to maturity (T4).

Enumeration, isolation and identification

For enumeration of PGPR population i.e. *Pseudomonas* sp., *Enterobacter* sp., *Azotobacter* sp. and *Acetobacter* sp. method described by Hu *et al.,* 2008 was used. Briefly, soil suspensions were prepared by shaking 1g rhizosphere soil sample in 9 ml Phosphate Buffer Saline (PBS). Resulting suspensions were spread onto the surfaces of appropriate selective media, and plates were incubated at 28ºC. Different selective media such as King's B medium supplemented with 3 antibiotics *i.e.,* Ampicillin (40 µg/mL), Cycloheximide (100 µg/mL) and Streptomycin (30 µg/mL) for *Pseudomonas* (King *et al.*, 1954; Sharma *et al.*, 2014a), Eosine Methylene Blue (EMB) agar for *Enterobacter* (Levine, 1918), Jensen's medium for *Azotobacter* (Jensen, 1954; Norris and Chapman, 1968), Acetobacter HiVeg Agar for *Acetobacter* and Pikovskaya's Agar for Phosphate-solubilizing bacteria (PSB) (Subba Rao, 1999) were used.

Phosphate solubilization

The ability of the bacterial isolates to solubilize mineral phosphate (P) was tested on Pikovskaya's medium as modified by Subba Rao and Shinta, 1963 in Subba Rao, 1999. The presence of clearing zone around bacterial colonies after overnight incubation was considered positive test P-solubilization.

Indole Acetic Acid (IAA) production

Briefly, Indole acetic acid (IAA) production was detected by the method of Bric *et al.*, 1991. After the growth of bacterial cultures for 72 hours on Tryptic Soy Agar (TSA) medium by spot inoculating the plates containing 10% TSA and 5 mM L-tryptophan the plates were overlaid with a nitrocellulose membrane and treated with Salkowski reagent (2% 0.5 M $FeCl_3$ in 35% perchloric acid) at room temperature for an hour for development of pink colour which indicated IAA production.

Ammonia (NH_3) production

For testing the production of ammonia by various bacterial isolates, a 5 µL of 24-hour grown culture was inoculated in 10 ml peptone water and incubated at 25ºC for 7 days. After incubation, the presence of ammonia (NH_3) was tested with 0.5 mL (1drop) of Nessler's Reagent which resulted in the development of yellow to brown colour (Cappuccino and Sherman, 1992).

HCN production

The production of HCN was tested by the method of Castric, 1977. A 10 µL of inoculum was placed in the centre of Tryptic Soy Agar (TSA) plates amended with Glycine 4.4 gL^{-1} and incubated at 28°C for 4 days. A Whatman filter paper No. 1 soaked in 2% Na_2CO_3 in 0.5% picric acid solution was placed on the top of the plate and plates were again incubated at 28ºC for 4 days. Development of yellow to orange-brown colour indicated HCN production.

Cellulase production

PGPR strains were observed for cellulase production by plating a 5 µL of overnight grown culture in nutrient broth on CMC agar (0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4.7H_2O$, 0.05% KCl, 0.2% carboxymethylcellulose (CMC) sodium salt, 0.02% peptone, and 1.7% agar). Plates were incubated at 28°C for 48 hours and then flooded with Gram's iodine (2.0 g KI and 1.0 g iodine in 300 mL distilled water) for 3 to 5 minutes. Cellulase activity was noticed by the presence of clear halos surrounding the colonies (Kasana *et al.*, 2008).

Siderophore production

The qualitative detection of siderophore production was carried out by the universal method of Schwyn and Neilands (1987) using blue agar plates containing the dye Chrome Azurol S (CAS). Briefly, bacterial isolates were inoculated in the centre of CAS agar and incubated at 28ºC for 3 days. Yellow to orange halos around the colonies on blue agar indicated the production of siderophore.

Chitinase activity

The chitinase activity of bacterial strains was tested on chitin agar plates (Wasli *et al.*, 2009) which were incubated at 30ºC for 5 days. Chitinase activity was identified by clear zone around the cells.

Protease activity

The protease activity (casein degradation) was determined using skim milk agar medium (Smibert and Krieg, 1994). Bacterial cells were spot inoculated onto the centre of skim milk agar plate and after 2 days of incubation at 28ºC, the proteolytic activity was identified by clear zone around the cells.

Antifungal activity

Antifungal activity was tested against phytopathogenic fungi on PDA plates according to the dual-culture technique on potato dextrose agar (PDA) medium (Cattelan *et al.*, 1999). Bacterial isolates were tested for *in vitro* antagonism towards two strains of fungal pathogens, namely, *Aspergillus niger* and *Fusarium moniliforme*. Isolates were inoculated on the plates by preparing bacterial lawn on the surface of PDA medium and PDA plugs of 7mm containing 48 hour old fungal mycelia were placed on the centre of each plate. After incubation of plates at 28ºC for 7 days they were examined for a zone of inhibition.

Statistical analysis

Statistical differences between numbers of bacteria (log_{10} c.f.u g^{-1} dry soil) from groundnut crop field at each growth stage were determined by independent-sample *t* tests at the 5% significance level. The treatment (effect of temperature), crop

growth stage (days after sowing) and treatment * crop growth stage interaction effects on the numbers ($\log_{10}$ c.f.u g^{-1} dry soil) of *Pseudomonas* spp., *Enterobacter* spp., *Azotobacter* spp. and *Acetobacter* spp. were tested and analysed by two-way analysis of variance (ANOVA) using a generalized linear model (GLM) procedure using SAS.

RESULTS

Our results revealed that elevation of +2-3ºC temperature from germination to maturity (T4) resulted in increase in population of PGPRs viz., *Pseudomonas* spp., *Enterobacter* spp., *Azotobacter* spp. and *Acetobacter* spp., by 4.39%, 6.81%, 6.81% and 3.29% respectively than the crop grown under ambient temperature conditions in groundnut rhizosphere.

The numbers of *Pseudomonas* spp., *Enterobacter* spp., *Azotobacter* spp. and *Acetobacter* spp. population were significantly different ($p < 0.001$) between the different temperature treatments (viz. T1-ambient temperature condition, T2-elevated temperature condition from germination to flowering, T3-elevated temperature condition from flowering to maturity and T4-elevated temperature condition from germination to maturity) as well as during all the various crop growth stages (Table 1.). The analysis of data for all treatments combined showed significant effect of temperature, crop growth stage and temperature * crop growth stage interaction for numbers of all categories of tested functional microorganisms.

Pseudomonas spp.

The population *Pseudomonas* spp. increased from $\log_{10}$ 5.6 c.f.u g^{-1} dry soil to $\log_{10}$ 6.3 c.f.u g^{-1} dry soil at 75 DAS (complete flowering) and then it started declining (Fig. 1 (a).). Similar trend was observed in other temperature treatments; however, the population in T4 and T2 showed 4.4% and 3.9% increase respectively during the same period. The number of *Pseudomonas* spp. was significantly higher in T4 than the other three treatments at all stages of crop growth and a consistent significant differences in the numbers of *Pseudomonas* spp. between T1 and T4 were observed at all stages. The number of *Pseudomonas* spp. in T4 was 8.1%, 4.3% and 4.6% higher than T1 at vegetative stage (30 DAS), complete flowering (75 DAS) and at maturity (105 DAS) respectively.

Table 1: Generalized linear model results of the overall effects of temperature treatment and growth stages on the number of *Pseudomonas* spp., *Enterobacter* spp., *Azotobacter* spp. and *Acetobacter* spp. in groundnut rhizosphere

Source of variation (by bacterial type)	df	F	P
Pseudomonas spp.			
Treatment	4	214.28	0.001
DAS	6	501.00	0.001
Treatment × DAS	24	5.11	0.001
Enterobacter spp.			
Treatment	4	348.18	0.001
DAS	6	479.45	0.001
Treatment × DAS	24	23.72	0.001
Azotobacter spp.			
Treatment	4	537.16	0.001
DAS	6	583.21	0.001
Treatment × DAS	24	29.70	0.001
Acetobacter spp.			
Treatment	4	209.26	0.001
DAS	6	199.01	0.001
Treatment*DAS	24	16.22	0.001

Enterobacter spp.

Significantly more *Enterobacter* spp. was detected in T4 than in the other three treatments at all crop growth stages. The number of *Enterobacter* spp. in T4 was 9.6%, 6.8% and 2.6% higher than ambient at vegetative stage (30 DAS), complete flowering (75 DAS) and at maturity (105 DAS) respectively (Fig. 1 (b).).

Azotobacter spp.

There were significantly fewer *Azotobacter* spp. in T1 than the other three treatments at pod development stage and at maturity. Significantly more *Azotobacter* spp. was detected in T4 than in the other three treatments at complete flowering. The difference in number of *Azotobacter* spp. in T1 and T3 was non-significant at vegetative stage, initial flowering and 50% flowering; however, it was significantly higher T3 than T1 at pod development stage and at maturity. The number of *Azotobacter* spp. in T4 was 6.2% and 5.7% higher than T1 and T2 respectively at complete flowering. (Fig. 1 (c).).

Acetobacter spp.

Similarly, at all stages of crop growth, significantly more *Acetobacter* spp. was detected in T4 than any of the other treatments (Fig. 1 (d).).

PGP activities

On the basis of cultural and morphological characteristics, a total of 62 isolates taken from five samplings in different stages of crop growth were grouped into *Pseudomonas*, *Enterobacter*, *Azotobacter* and *Acetobacter* spp. In the present investigation 20 isolates of *Pseudomonas* spp., 17 isolates of *Enterobacter* spp., 13 isolates of *Azotobacter* spp. and 12 isolates belonging to *Acetobacter* spp. were screened for *in vitro* plant growth promoting (PGP) activities during various stages of crop growth.

In our experiment, phosphate solubilizing isolates showed clear zone of hydrolysis on agar plates and according to their zone diameter the isolates were marked as "+,++,+++". Solubilization of phosphate was exhibited by all isolates of *Enterobacter* spp. 74.19% of the total isolates were phosphate solubilizers of which *Pseudomonas* spp. and *Acetobacter* spp. constituted 85% and 75% respectively. The increase of temperature +2-3ºC above ambient did not result in any significant change in the phosphate solubilizing activity among different test isolates (Plate 1 (a).).

Production of IAA was indicated as the development of pink-coloured auxin complex (Plate 1 (b).). 74.19% of the total isolates were able to produce IAA. Results revealed that the isolates of *Acetobacter* spp. were strong IAA producers (100%) followed by *Enterobacter* spp. (82.4%) and *Azotobacter* spp. (69.2%), whereas, few isolates of *Pseudomonas* spp. (55%) were test positive for IAA. *Pseudomonas* spp. was sensitive for IAA production at 50% flowering (60 DAS) for all treatments, however, no significant difference among the isolated strains with respect to the effect of temperature in different treatments

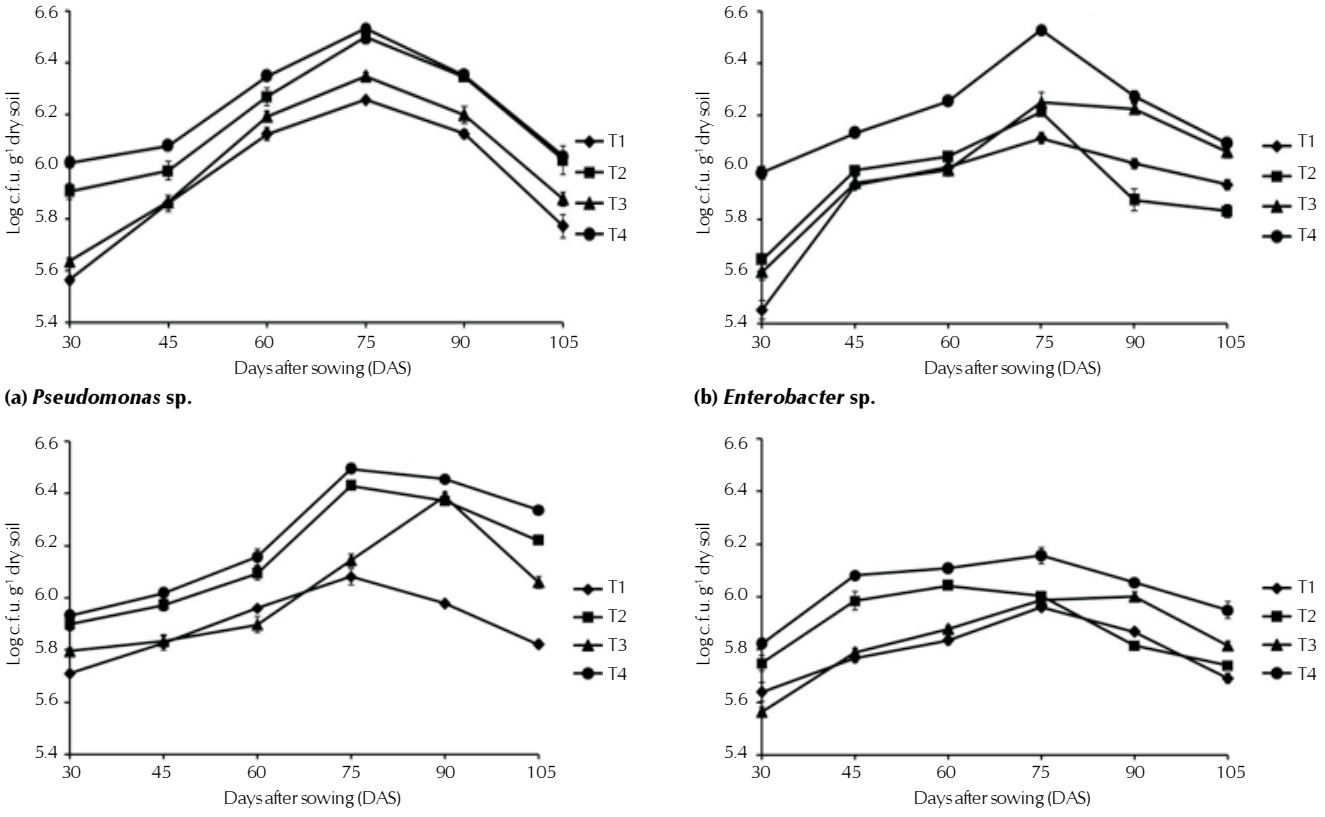

Figure 1: PGPR population dynamics at different stages of crop growth (a) *Pseudomonas* spp., (b) *Enterobacter* spp., (c) *Azotobacter* spp. and (d) *Acetobacter* spp. in groundnut (B-95) rhizosphere grown under ambient T1 (germination to maturity: ambient temperature) and above ambient temperature such as T2 (germination to flowering + 2-3ºC), T3 (flowering to maturity + 2-3ºC) and T4 (germination to maturity + 2-3ºC) treatments

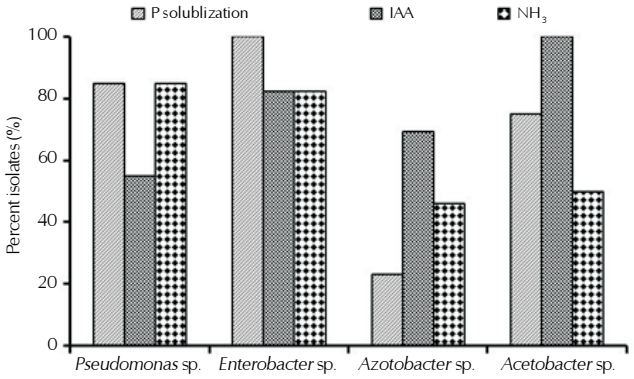

Figure 2: Direct PGP activities of test isolates

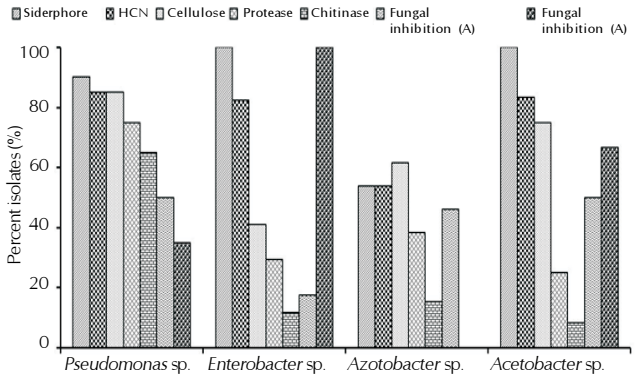

Figure 3: Indirect PGP activities of test isolates

could be seen.

NH_3 production was characterized by the formation of by yellow-brown precipitate (Plate 1 (c).). NH_3 production did not show any significant difference in the isolated PGPR strains during various sampling stages. 69.35% of the total isolates were positive for NH_3 production. *Pseudomonas* spp. were strong producers with 85% of isolates as test positive followed by *Enterobacter* spp. (82.4%).

No significant effect of temperature treatments was observed in siderophore production between isolates of different strains at different sampling stages. 87.1% of the total isolates displayed siderophore production (Plate 1 (d).), out of which all isolates of *Enterobacter* spp. and *Acetobacter* spp. showed siderophore production followed by *Pseudomonas* spp. (90%) and *Azotobacter* spp. (53.8%).

HCN production was revealed by the formation of red colour on filter paper soaked in picric acid. HCN production was exhibited in 77.42% of all the isolates tested for HCN

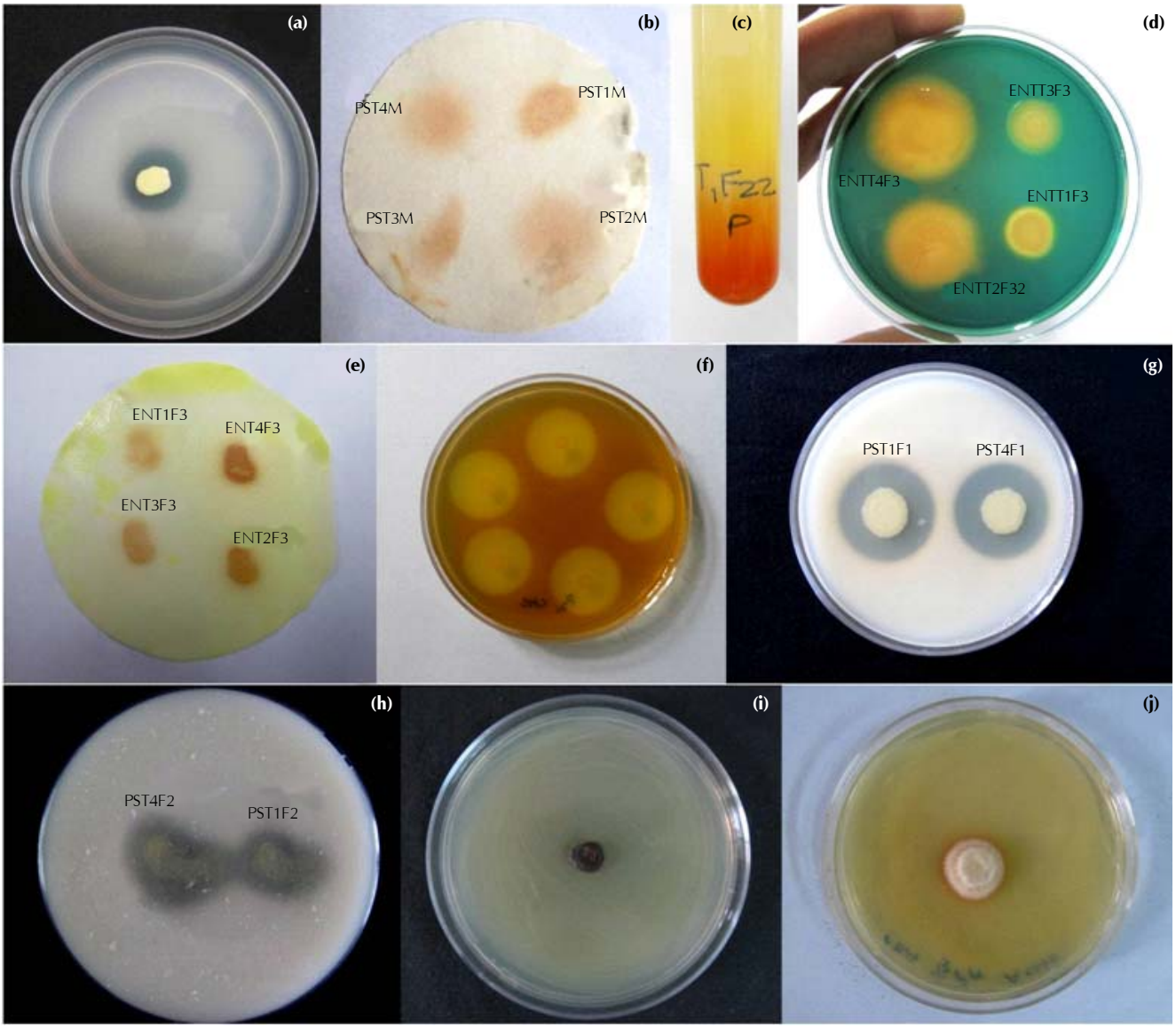

Plate 1: (a) Phophate solubilisation on Pikovakaya's agar medium by stain by PST1F2, (b) IAA production by different Enterobacter strains, (c) Ammonia production in peptone water by Pseudomonas strain PST1F22,)d) Siderphore production on CAS agar medium by different strains of Enterobatcter, (e) Cyanide production by different Enterobater strains, (f) Cellulase production on CMC ager medium by *Pseudonas* strain PST1F3(g) Protease activity on skim milk agar medium by Pseudomonas strains PST1F1 and PST4F1, (h) Chitinase activity on Chitinase ager medium by strains Pseudomonas PST4F2 and PST1F2, (i) Fungali inhibition of Asergillus niger by Pseudonas strains PST4F2, (J) Fungal inhibition of Fusariforme by Acetobacter strain ACT3P

production (Plate 1(e).). 85% of *Pseudomonas* spp. were HCN positive followed by *Acetobacter* spp. (83.3%), *Enterobacter* spp. (82.4%) and *Azotobacter* spp. (53.8%). HCN production did not vary significantly with temperature treatments in all isolates at all crop growth stages.

Cellulase positive isolates showed distinct, clear, and prominent zones of clearance around the colonies showing cellulase production with bluish-black colouration in the non-hydrosylated part of the medium (Plate 1 (f).). Cellulase production was demonstrated by 66.13% of the test isolates out of which maximum production was displayed in *Pseudomonas* spp. (85%) followed by *Acetobacter* spp. (75%), *Azotobacter* spp. (61.5%) and *Enterobacter* spp. (41.2%). Cellulase production was affected by temperature at various growth stages but there was no specific change that could be traced, however cellulase production in ambient temperature was present but it was absent in elevated temperature treatments.

Pseudomonas spp. were strong at exhibiting protease activity with 75% of isolates as test positive (Plate 1 (g).). Out of 45.16% of total isolates showing protease activity 38.5% was constituted by *Azotobacter* spp., 29.4% by *Enterobacter* spp. and 25% by *Acetobacter* spp. It was revealed from the experiment that although protease production was not affected by temperature treatments it was significantly affected by growth stage in different bacterial strains.

Chitinase activity was revealed only in 29.03% of the test isolates and did not vary with temperature treatments although chitinase activity did vary among the strains of different bacterial species (Plate 1 (h).). *Pseudomonas* spp. showed the highest chitinase activity with 65% of isolates positive for chitinase activity during different sampling stages at different temperature treatments followed by *Enterobacter* spp. (11.8%), *Azotobacter* spp. (15.4%) and *Acetobacter* spp. (8.3%). *Acetobacter* strains were affected maximum due to elevated temperature conditions.

Of the 62 isolates tested for antifungal activity 25 and 32 strains could display antagonism for *A. niger* and *F. moniliforme* respectively. The antifungal activity of strains tested varied with the two fungal strains. Isolates of *Enterobacter* spp. showed complete inhibition (100%) for *F. moniliforme* (Plate 1 (i).), while only 17.6% of its isolates showed inhibition for *A. niger* (Plate 1 (j).). *Azotobacter* spp. strains could not inhibit *A. niger*, however 46.2% of its isolates could inhibit *F. moniliforme*. 50% and 35% of *Pseudomonas* spp. could exhibit inhibition of *A. niger* and *F. moniliforme* respectively. Fungal inhibition activity did not vary with temperature treatments but differed largely because of fungal strain.

DISCUSSION

Plant rhizosphere has been shown to be a preferred ecological niche for different types of soil microorganisms due to rich nutrient availability and is thus considered a dynamic and versatile ecological environment of distinct microbes–plant interactions for harnessing essential micro- and macro-nutrients from a limited nutrient pool (Jeffries *et al.*, 2003). PGPR colonize plant roots and exert beneficial effects on plant growth and development by a wide variety of mechanisms. To be an efficient PGPR, bacteria must be able to colonize roots because bacteria need to establish itself in the rhizosphere at population densities sufficient to produce beneficial effects. The exact mechanism by which PGPR stimulate plant growth is not clearly established, although several hypotheses such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of mineral nutrient uptake are believed to be involved (Khan *et al.*, 2009; Zaidi *et al.*, 2009).

In the present investigation some PGPR viz. *Pseudomonas* spp., *Enterobacter* spp., *Azotobacter* spp. and *Acetobacter* spp. were enumerated under different temperature treatments. It was observed that there was greater numbers of PGPR in T4 than any other treatment irrespective of PGPR genera. The reason behind these results can be attributed to the fact that with an increase in temperature, as has been predicted under future climate conditions, root exudation of organic C also increases (Uselman *et al.*, 2000). Their results showed that as temperature increases as has been predicted under future climate conditions, root exudation of organic C also increases by a factor of 1.7. This implies that exudation of organic C may be an active metabolic process, stimulated by the increase in temperature. Zogg *et al.* (1997) found that microbial respiration increased dramatically with soil warming due to an apparent increase in the pool size of C metabolized by soil microbes at higher temperatures. One plausible mechanism for this response can be due to a temperature-induced shift in microbial community composition, wherein dominant populations at higher temperatures have the ability to metabolize substrates that are not utilized by members of the microbial community at lower temperatures. It is widely recognized that certain groups of soil microorganisms are well adapted to particular temperature regimes. Furthermore, soil microbes can vary considerably in their affinity for different substrates. Therefore, if temperature elicits changes in community composition, the dominant populations may be favoured at higher temperatures which may metabolize the increased amount of substrates and colonize the rhizosphere in increased numbers. As nutrient availability is the primary determinant of microbial population size in the rhizosphere (Zaidi *et al.*, 2009), probably there may be increase in release of root exudates, which resulted in increased population of PGPR. Presence of high number of PGPR in the groundnut crop being grown under elevated temperature conditions at different stages of crop growth could be playing a role in the improved nutrition to these plants, thereby conferring vigour to the groundnut plants. These observations are in direct conformation with the report of Dey *et al.*, 2004, who also reported the positive effect of PGPR on groundnut.

Phosphorus (P), an essential element for plant growth and productivity and the second important plant growth-limiting nutrient after nitrogen, is abundantly available in soils in both organic and inorganic forms (Khan *et al.*, 2009). Only a few rhizobial strains have the capability to solubilize non-solubilizing phosphorous in soil and as a result increase plant growth and productivity (Rodriguez and Fraga, 1999). Of the enumerated PGPR population 62 PGPR isolates were evaluated for their PGP activities and 74.19% of test isolates were found to produce clear yellow zones around the inoculums on Pikovskaya's medium containing insoluble phosphate. Ahemad and Khan (2010) have reported that the performance of Phosphate solubilising bacteria (PSB) is specially affected by environmental factors especially under stress conditions. Whereas Bhattacharyya and Jha (2012) have reported the bacterial genera like *Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium* and *Serratia* as the most significant phosphate solubilizing bacteria, Mittal *et al.* (2008) have confirmed that these PSB not only provide the P to plants, but they also stimulate the plant growth by synthesizing the plant growth promoting substances, nitrogen fixation as well as by enhancing the availability of the trace elements. In another study by Sharma *et al.* (2014b) *Pseudomonas* isolates have been shown to produce phosphate solubilizing activity in the range of 199.5 to 413.4 ig/mL available inorganic phosphate (Pi) and siderophore production in the range of 20-21 mm in plate assay and 67.27 %SU in liquid assay.

The results for IAA production are in consistent with the findings of several research workers such as Ahmad *et al.* (2008) who have reported the production of IAA in some *Pseudomonas* and *Azotobacter* strains with different tryptophan concentrations with maximum production of IAA. In another study by Begam *et al.* (2014) *Azotobacter* strains from tomato rhizosphere have been reported to produce IAA (1.196-1.582 ηg/mL).

Ammonification is an important step in the transformation of organic nitrogen to ammoniacal form and is known to enhance soil nitrogen content by the ammonifying character of the PGPR isolates. Although Dey *et al.*, 2004 have suggested insignificant involvement of *P. fluorescens* strains in ammonification trait our results are in tune with Ahmad *et al.* (2008) who have have shown similar results of NH_3 production with *Pseudomonas* and *Azotobacter* and *Bacillus* strains isolated from the rhizospheric soil of different crops (mustard, berseem, wheat, sugarcane, brinjal, onion, cauliflower, cabbage and chick pea and *Mesorhizobium* from nodules of chickpea.

Iron is a critical nutrient for almost all forms of life. In the soil, iron occurs principally as Fe^{3+} and is likely to form insoluble hydroxides and oxyhydroxides, thus making it generally inaccessible to both plants and microorganisms (Rajkumar *et al.*, 2010). Siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation (Indiragandhi *et al.*, 2008). Thus siderophore production was another important PGP trait of the test isolates. 87.1% of test isolates were found positive for siderophore production which is in consistent with the results demonstrated by many researchers including Sharma *et al.* (2014a).

HCN and antifungal activity are indirect plant growth promoting activities. Bhattacharyya and Jha (2012) have reported rhizobacteria producing antifungal metabolites like, HCN, phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol, pyoluteorin, viscosinamide and tensin and Ahmad *et al.* (2008) have reported PGPR isolates belonging to *Azotobacter*, fluorescent *Pseudomonas*, *Mesorhizobium* and *Bacillus* to show antifungal activity against *Aspergillus* sp., *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia bataticola*. Our results conform with these studies.

Sufficient literature could not be found on the effect of temperature on the various PGP traits of the isolates. Hence not much can be said about it; however our results showed that there was no loss or gain of any PGP trait among the selected test isolates as an effect of elevated temperature conditions in the present and future climate change context. This could be a novel finding in this respect.

Hence, it can be concluded from the present investigation that, since most of the isolated strains were able to exhibit more than three PGP traits and their presence in the groundnut crop being grown under elevated temperature conditions at different stages of crop growth, gives a clear indication that high numbers of PGPR could be playing a role in the improved nutrition to these plants, thereby conferring vigour to the groundnut plants. In addition to this, the isolates which are strong at plant growth promoting activities can be used as biofertilizers for enhancing crop growth after further research.

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