Effects of exogenously applied plant growth regulators in combination with PGPR on the physiology and root growth of chickpea (*Cicer arietinum*) and their role in drought tolerance

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**ABSTRACT**

Both the plant growth promoting rhizobacteria (PGPR) and plant growth regulators (PGR) exert beneficial effects on plant growth even under stress, but combined effect of both of them has not been evaluated yet. Present investigation was aimed to determine the responses of chickpea varieties (differing in drought tolerance) to 3 PGPR viz. *Bacillus subtilis*, *Bacillus thuringiensis* and *Bacillus megaterium* and PGR (SA and Putrescine) on physiology of chickpea grown in sandy soil. The PGR, Salicylic acid (SA) and Putrescine (Put) were sprayed on the seedling 20 days after germination. Results revealed, synergistic effects of PGPR and PGR on chlorophyll, protein and sugar contents. Addition of PGR to PGPR inoculated plants assisted the plant in osmoregulation and amelioration of oxidative stresses and in induction of new proteins. Combined application of PGPR and PGR decreased lipid peroxidation more effectively but increased the leaf area. It is inferred that PGPR and PGR work synergistically to promote growth of plants under moisture and nutrient deficit condition of sandy soil. Since, SA induces Systemic Acquired Resistance (SAR) in plants hence the addition of SA along with PGPR may render the plant more productive and better tolerant to diseases/pathogen attack.

**Introduction**

Abiotic stresses are the most damaging for plants, among these the most destructive that affect the plants from physiological to molecular level is drought stress that limiting the growth and yield of crop plants (Khan et al. 2018; Pereira and Chaves 1995). It is estimated that drought stress may cause a 50% loss in crop plants (Kasim et al. 2013). Drought disturbs the water potential and turgor of the plants sufficiently, to inhibit with normal functioning of the plant (Hsiao 2000). Drought also induces secondary stress e.g. oxidative stress, that can cause damage to proteins, lipids and nucleic acid in plants and can initiate lipid peroxidation (Hendry 2005; Nair et al. 2008). Legumes, especially chickpea is an important crop plant of Pakistan that covers, 1028.90 thousand hectares i.e. 4.3% of the total cultivated area (Shah et al. 2007). Research is being diverted to formulate strategies to manage drought stress either by developing drought tolerant varieties or by shifting the crop calendars (Venkateswarlu and Shanker 2009) however, these methods are not economical and are time consuming. Recent studies postulates that PGPR in association with PGR can help plants to cope with drought stress in much better way.

Plants inoculated with PGPR strains, flourish well under drought stress in arid and semi-arid regions (Marulanda et al. 2007). Plant growth regulators play vital role in the plant developmental processes (Asgher et al. 2015). Salicylic acid (SA), a monohydroxybenzoic acid and Putrescine (Put), a polyamine play key role in the regulation of plant growth and development as they trigger plant responses to various stresses (Duan et al. 2008; Miura and Tada 2014).

The present study was therefore, aimed to evaluate the role of three PGPR strain and two PGR, salicylic acid and putrescine applied alone and in association with PGPR on the performance of chickpea grown in sandy soil.

**Materials and methods**

The experimental work was carried in the green house of the department of Plant Sciences, Quaid-i-Azam University Islamabad, in chickpea growing season 2015–2016. Two chickpea varieties Punjab Noor-2009 (drought sensitive) and 93127 (drought tolerant), were obtained from Ayub Agricultural Research Institute, Faisalabad and were sown in plastic pots measuring 30 × 40 cm² and filled with sandy soil. Bacterial colonies were isolated from the rhizospheric soil of chickpea grown in Cholistan desert of Pakistan and were named as P1 P2 and P3 which were later sequenced for 16S rRNA as *Bacillus subtilis* (P1), *Bacillus thuringiensis* (P2) and *Pseudomonas fluorescens* (P3). The experiment was laid out as Completely Randomized Design (CRD) with four replications.

The treatments made were:

- T1- P1 inoculation,
- T2- P1 inoculation in association with SA and Put

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**T₃**-Coinoculation of P2 and P3, **T₄**-Coinoculation of P2 and P3 + SA and Put

**T₅**-Coinoculation of P1, P2 and P3, **T₆**-Coinoculation of P1, P2 and P3 in association with SA and Put, **T₇**- Plants sprayed with SA only (150 mg/L),

**T₈**-Plants sprayed with Put only (150 mg/L), **T₉**- Combined treatment of SA and Put,

**T₁₀**-Uninoculated and untreated stress control, **T₁₁**- Irrigated Control

**Where**-P1: *Bacillus subtilis* P2: *Bacillus thuringiensis* P3: *Bacillus megaterium*

**Sterilization of seeds**
The seeds were sterilized with 70% ethanol, followed by soaking in chlorox (10%) for 10–15 min. and subsequently washed with autoclaved distilled water.

**Method of inoculation**
The Luria Bertani (LB) broth was inoculated with fresh (24 h old) bacterial culture. The inoculated LB broth was incubated in shaker for 48 h at 27°C followed by centrifugation at 10000 rpm for 10 min. The supernatant was removed and the pellet was suspended in distilled water and the optical density (at 660 nm) was adjusted to 1. Seeds were soaked for 3 h in the broth culture thus prepared and were sown in the field.

**Description of isolated PGPR**

**Morphology of bacterial colony**
Bacterial strains were identified on the basis of colony and cell morphology. Picovskaya’s media was used for overnight growth of bacterial isolates (Picovskaya 1948). The color and shape of the colonies was recorded after 24 h.

**Catalase and oxidase test**
Test for catalase and oxidase enzymes was performed by the method of MacFadden (1980) and Steel (1956).

**Extraction of bacterial DNA**
The bacteria DNA was extracted following the method of Chen and Kuo (1993).

**PCR and sequence analysis (16S rRNA)**
The method of Weisburg et al. (1991) was followed for the amplification of genomic DNA of bacterial cell. The nucleotide sequence of fd1 primer was AGAGTTGATCCTGGCATCG and that of rd1 was AGGAGGTGATCCAGC. Electrophoresis for PCR products was done on 1.2% gel using DNA ladder of 1 kb. Ethidium bromide was used for staining gel and the product was examined under UV transilluminator (S. N. 765/64069, Bio RAD, Italy).

**P-solubilisation index (PSI)**
P-solubilisation index of bacterial colony was calculated following Picovskaya (1948)

**Antibacterial and antifungal activities**
An agar well diffusion method was used for the determination of antibacterial activity (Navarro et al. 1996), whereas for antifungal activity, agar tube dilution method of Washington and Sutter (1980) was followed.

**Biochemical analyses of chickpea**

**Proline content (μg)**
Proline content of leaves was measured by the method of Bates and Waldern (1983).

**Chlorophyll content**
The chlorophyll content of chickpea leaves was determined by using SPAD chlorophyll meter (Spad 502 plus plus, S.No.2000147 Konica Minolta, Japan).

**Leaf protein contents**
For examination of leaf protein content the method of Lowry et al. (1951) was followed.

**Sugar estimation**
Sugar content was estimated by the method of Dubey and Singh (1999).

**Lipid peroxidation (MDA)**
The lipid peroxidation was determined by calculating the amount of Malondialdehyde (MDA) formed by thiobarbituric acid (TBA) reaction as defined by Li et al. (2000). The MDA concentration was determined by the following formula:

\[
C_{MDA} \text{ (μmol L}^{-1}) = \frac{6.45(A_{532} - A_{600})}{0.56 A_{450}},
\]

from which the absolute concentration (mmol g⁻¹ FW) of malondialdehyde was calculated.

**Assay for antioxidant enzymes**
Fresh leaf tissue (0.5 g) was used for the determination of antioxidant enzymes activity. The fresh leaf tissue was grinded, in 5 ml of 50 mM phosphate buffer in an ice bath. The mixture was centrifuged for 20 min. at 13000 rpm at 4°C. The supernatant was used for different enzyme assays.

For peroxidase (POD) determination, the modified method of Gorin and Heidema (1976) was followed. Ascorbate peroxidase (APOX) activity was determined as described by Asada and Takahashi (1987). Catalase (CAT) activity was estimated following the method of Chandlee and Scandalios (1984).

**Root length and root area**
Average length of five randomly selected roots was recode and root area was also measured by using root law.

**Statistical analysis**
The experiment was conducted with four replicates. The data was analysed by analysis of variance (ANOVA) followed by Least square difference (LSD) at \( P = 0.05 \) using Statistica version 8.1 and the results were expressed as mean ± standard error (SE) for each treatment.
Results

Phosphate solubilisation index (PSI)

Bacillus subtilis was the most efficient phosphorous solubiliser with a phosphorus solubilisation index (PSI) of 2.8. The PSI values for Bacillus thuringiensis and Bacillus megaterium were 2.5 and 1.7, respectively (Table 1).

Antibacterial and antifungal activities of PGPR isolates

Bacillus subtilis, Bacillus thuringiensis and Bacillus megaterium showed activities against Staphylococcus aureus, Klebsiella pneumonia and Escherichia coli (Table 2).

Maximum inhibition (73%) of Helminthosporium sativum was observed due to Bacillus thuringiensis whereas, Bacillus subtilis significantly inhibited the growth of Fusarium solani (84%) followed by the Bacillus thuringiensis (83%) (Table 2).

Physiological and biochemical analyses of plants

Chlorophyll content

The chlorophyll content was increased in all the treatments over the untreated control (T10) however, the values were lower than irrigated control (T11) except for T6 (coinoculation of P1, P2 and P3 in combination with SA and Put). In T4 (coinoculation of P2 and P3 with SA and Put), T5 (coinoculation of P1, P2 and P3) and T6 (coinoculation of P1, P2 and P3 in combination with SA and Put), the chlorophyll content of sensitive variety was increased over tolerant variety. For sensitive variety, Put (T8) alone was less effective than SA (T7); whereas, the combined treatment of SA and Put was more effective (Figure 1).

Leaf proline content

The leaf proline content was increased in all the treatments over the stress control (T10) in both the varieties. The proline content in tolerant variety was significantly higher over irrigated control. The SA (T7) and putrescine (T8) treatments were more responsive in sensitive variety. The proline content was significantly lower in T6 (coinoculation of P1, P2 and P3 in combination with SA and Put) as compared with T5 (coinoculation of P1, P2 and P3) and untreated control. T7 (SA) and T8 (Put) were more effective than T9 (SA+Put) in both the varieties (Figure 2).

Leaf protein content

Treatments T6 (coinoculation of P1, P2 and P3 in combination with SA and Put) and T1 showed maximum accumulation and significantly increased (53% and 28%) the leaf protein content as compared to untreated control (T10). The leaf protein content was also higher in T7 (SA) over uninoculated control in the sensitive variety. Put had reduced the protein accumulation in the sensitive variety more than 50% of T7. The PGR and PGPR act synergistically in accumulation of protein (Figure 2).

Table 1. P-solubilizing activity of isolated bacterial strains.

| Isolates        | Halozone Diameter (mm) | P-solubilization index |
|-----------------|------------------------|------------------------|
| Bacillus subtilis | 1.5                    | 2.8                    |
| Bacillus thuringiensis | 1.2                | 2.5                    |
| Bacillus megaterium | 0.8                  | 1.7                    |

Measurement was made after 7 days of inoculation.

Table 2. Antibacterial and Antifungal activities of selected PGPR strains.

| Isolates       | Anti-Bacterial Activities | Antifungal Activities |
|----------------|--------------------------|-----------------------|
|                | Staphylococcus aureus     | K. pneumoniae         | E. coli | H. sativum | F. solani |
| Bacillus subtilis | +                       | +                     | +       | 66%       | 84%       |
| Bacillus thuringiensis | +                    | +                     | +       | 73%       | 83%       |
| Bacillus megaterium | +                     | +                     | +       | 51%       | 74%       |

Figure 1. Chlorophyll content in the leaves of chickpea drought sensitive (S) and tolerant (T) varieties grown in sandy soil. Data are means of four replicates along with standard error bars. Different letters indicating significant difference within treatments. T1- P1 inoculation; T2- P1 inoculation in association with SA and Put; T3- Coinoculation of P2 and P3; T4- Coinoculation of P2 and P3 + SA and Put; T5- Coinoculation of P1, P2 and P3; T6- Coinoculation of P1, P2 and P3 in association with SA and Put; T7- Plants sprayed with SA only (150 mg/L); T8- Plants sprayed with Put only (150 mg/L); T9- Combined treatment of SA and Put; T10- Uninoculated and untreated stress control; T11- Irrigated Control.
Leaf sugar content

The leaf sugar content was increased in all the treatments with respect to stress control (T10) but the values were lower (50%) than irrigated control. Tolerant and sensitive variety showed similar response. T1 (P1 inoculation), T2 (P1 in combination with SA and Put) and T7 (SA), were at par for both the tolerant and sensitive varieties. T5 (coinoculation of P1, P2 and P3) showed maximum increase (69%) in both the sensitive and tolerant varieties over stress control (T10). Maximum decrease in leaf sugar content was observed in T2 (P1 inoculation) and T6 (coinoculation of P2 and P3 in association with SA and Put; T9 - combined treatment of SA and Put) treatments except in T8 (Put) and T9 (SA and Put). SA (T7) was more effective in reducing the leaf sugar content as compared to stress control (T10). Results revealed significant decreases in the leaf sugar content in the leaves of plants inoculated with PGPR or treated with PGR alone or in combination as compared to stress control (T10). In general, the tolerant variety showed higher leaf sugar content as compared to sensitive variety in all the treatments except in T5 (coinoculation of P1, P2 and P3) and T4 (coinoculation of P2 and P3 in combination with SA and Put) had equal % decrease in leaf sugar content. PGR alone were more effective in reducing the lipid peroxidation when applied in combination (T9) (Figure 3).

Antioxidant enzymes activity

All the inoculated plants showed lower values for catalase activity as compared to stress control (T10). T8 was most efficient in reducing (34% and 40%) the catalase activity in both the sensitive and tolerant varieties followed by the combined treatment of SA and Put (32% and 37%). Results revealed significant decreases in ascorbate peroxidase activity in the leaves of plants inoculated with PGPR or treated with PGR alone or in combination as compared to stress control (T10). In general, the tolerant variety showed higher ascorbate peroxidase activity as compared to sensitive variety in all the treatments except in T5 (coinoculation of P1, P2 and P3) and T4 (coinoculation of P2 and P3 in combination with SA and Put) had equal % decrease in ascorbate peroxidase activity in both the tolerant and sensitive varieties. Combined treatment of PGPR and PGR were more effective in reducing the lipid peroxidation as compared to sensitive variety in all the treatments except in T5 (coinoculation of P1, P2 and P3) and T4 (coinoculation of P2 and P3 in combination with SA and Put) had equal % decrease in lipid peroxidation. PGR alone were more effective in reducing the ascorbate peroxidase activity as compared to sensitive variety in all the treatments except in T5 (coinoculation of P1, P2 and P3) and T4 (coinoculation of P2 and P3 in combination with SA and Put) had equal % decrease in ascorbate peroxidase activity in both the tolerant and sensitive varieties. Combined treatment of PGPR and PGR were more effective in reducing the ascorbate peroxidase activity than PGPR alone (Figure 4).

The POD activity was decreased in all the treatments except for T1 as compared to stress control (T10). Maximum decrease (39%) in POD activity was recorded in T6 = T4 in
both the sensitive and tolerant varieties though, the values were higher (43% and 17%) than irrigated control (T11). All the PGPR bioinoculants and SA treatments had higher POD activity in both the varieties but Put alone or in combination with SA or PGPR, decreased the POD activity. T6 (coinoculation of P1, P2 and P3 in combination with SA and Put) showed maximum decrease (60% and 53%) in superoxide dismutase (SOD) activity and T6 = T11. Combined treatment of SA and Put (T9) was less effective as compared to SA and Put used alone (Figure 5).

Leaf area

The leaf area was increased in all the treatments over stress control. Maximum increase (59% and 56%) in leaf area was recorded in T9 (combined treatment of SA and Put; T9- Coinoculation of P2 and P3; T9- Coinoculation of P2 and P3 + SA and Put; T9- Coinoculation of P1, P2 and P3; T9- Coinoculation of P1, P2 and P3 in association with SA and Put; T9- Plants sprayed with SA only (150 mg/L); T9- Plants sprayed with Put only (150 mg/L); T9- Combined treatment of SA and Put; T10- Uninoculated and untreated stress control; T11- Irrigated Control).

Figure 4. Catalase and APOX activities in the leaves of chickpea drought sensitive (S) and tolerant (T) varieties grown under sandy soil. Data are means of four replicates along with standard error bars. Different letters indicating significant difference within treatments. T1- P1 inoculation; T2- P1 inoculation in association with SA and Put; T3- Coinoculation of P2 and P3; T4- Coinoculation of P2 and P3 + SA and Put; T5- Coinoculation of P1, P2 and P3; T6- Coinoculation of P1, P2 and P3 in association with SA and Put; T7- Plants sprayed with SA only (150 mg/L); T8- Plants sprayed with Put only (150 mg/L); T9- Combined treatment of SA and Put; T10- Uninoculated and untreated stress control; T11- Irrigated Control.

Root length and root area

Root length was increased in all the treatments over stress control. Maximum increase (75% and 68%) in root length was recorded in T6 (coinoculation of P1, P2 and P3 in combination with SA and Put) for both the sensitive and tolerant varieties followed by T9. Treatments T2 and T3 were also effective for increasing root length. P1 inoculation in combination with SA and Put (T2) was more effective than P1 inoculation alone (T1). Sensitive variety was more responsive to PGPR inoculation. Put (T8) was more effective than SA (T7) in sensitive variety The combined treatment of SA and Put (T9) was more effective than P1 inoculation in association with SA and Put (T8) alone (Figure 6).

Root area was significantly enhanced in the combined treatment of PGPR and PGR over stress control (T10) and irrigated control. Maximum increase (79% and 73%) in

Figure 5. POD and SOD activities in the leaves of chickpea drought sensitive (S) and tolerant (T) varieties grown under sandy soil. Data are means of four replicates along with standard error bars. Different letters indicating significant difference within treatments. T1- P1 inoculation; T2- P1 inoculation in association with SA and Put; T3- Coinoculation of P2 and P3; T4- Coinoculation of P2 and P3 + SA and Put; T5- Coinoculation of P1, P2 and P3; T6- Coinoculation of P1, P2 and P3 in association with SA and Put; T7- Plants sprayed with SA only (150 mg/L); T8- Plants sprayed with Put only (150 mg/L); T9- Combined treatment of SA and Put; T10- Uninoculated and untreated stress control; T11- Irrigated Control.
root area was recorded in T₅ (coinoculation of P₁, P₂ and P₃) followed by T₆ (coinoculation of P₁, P₂ and P₃ in combination with SA and Put). Treatment T₁ (P₁ inoculation) was at par in both the sensitive and tolerant varieties whereas, T₁ = T₄ and T₅ = T₆ for tolerant variety. Among the PGR treatments, combined treatment of SA and Put was more effective and enhanced the root area by 71% and 48% in sensitive and tolerant varieties respectively. Both the PGR treatments were more effective in sensitive variety than tolerant variety (Figure 7).

**Discussion**

Sandy soils have lower water holding capacity and also retain lesser nutrients. PGPR are the main components of soil biodiversity and play a key role in enhancing soil nutrients and moisture content as they colonize the rhizosphere of plants and produce various substances including exopolysaccharides, phytohormones, aminocyclopropane-1-carboxylate deaminase, induce accumulation of osmolytes, antioxidant enzymes and adopt root morphology to drought stress (Vurukonda et al. 2016). PGPR appear to act synergistically to augment the chlorophyll content of the leaves and the sensitive variety was more responsive to PGR and PGPR. SA alone had greater values for chlorophyll content than P₁ inoculation (T₁). It had been previously reported that SA and Putrescine significantly enhance the chlorophyll content in several crop plants (Zhang et al. 2009; Rivas-San Vicente and Plasencia 2011; Durmuş and Bekircan 2015). The stimulatory role of PGPR on leaf chlorophyll content had been reported previously (Vafadar et al. 2014; Fahad et al. 2015).

Proline accumulation occurs in plants under various abiotic stresses (Ashraf and Foolad 2007). Proline play a key role in osmoregulation and bioenergetics of cell (Ambikapathy et al. 2002; Pandhare et al. 2009). The combined treatment of all 3-PGPR and 2-PGR (T₆) had significantly reduced the proline content, which may be attributed possibly to PGR and PGPR induced regulation of osmotic balance and maintenance of the bioenergetics of the cell. Accumulation of soluble sugars had profound effect on osmo-protection
and scavenging of free radicals (Datta and Kulkarni 2014). Instead the PGPR bioinoculants enable the plant to adjust osmotic potential by augmenting sugar content in the leaves of plants grown in sandy soil. Sandhya et al. (2010) demonstrated the antagonistic effects of drought stress on plant growth under uninoculated condition may be credited to reduction in the content of starch and sugar. *Serratia marcescens* CDP-13 enhanced salinity tolerance of chickpea by modulating the concentrations of different osmo-protectants including sugars (Singh and Jha 2016). SA treatments had profound effects on sugar content. The role of Put in accumulation of sugars in leaves of plants under stress condition had also been reported previously (Jiang et al. 2012; Baniasadi et al. 2015). Similar, results had also been reported by Jha et al. (2011), that proline accumulation increased with salinity but decrease in plants inoculated with *P. pseudoalcaligenes* and *B. pumilus* alone or in combination. The role of PGR on proline content had also been reported earlier (Khan et al. 2013; Gupta and Huang 2014). Su and Bai (2008) demonstrated negative correlation between accumulation of proline and endogenous Put content in soyabean leaves grown under stress condition.

Water soluble, stress proteins play an important role in plants to tolerate stress (Wahid 2007). Enhanced leaf protein content in legumes following *Rhizobium* and PGR application have previously been reported (Figueiredo et al. 2008). The SA treatment was much responsive in sensitive variety. SA had been reported to ameliorate salt stress induced decrease in the protein content of tomato leaves (Zahra et al. 2011). Put application prevented the degradation and reduction of proteins under moisture stress (Ullah et al. 2012).

Lipid peroxidation (measured as MDA content) is one of the biochemical marker to drought stress. The lipid peroxidation showed significant decrease in all the PGPR inoculated and PGR treated plants. Jha and Subramanian (2014) found that inoculation with a single PGPR reduced the lipid peroxidation by 1 time while, combination of 2 PGPR reduced the content up to 1.6 time under salt stress condition. Koc (2015) also reported the negative impacts of plant growth promoting bacteria and arbuscular mycorrhizal fungi on lipid peroxidation and total phenolics of strawberry when grown under stress condition. The exogenous application of SA and Put reduced the lipid peroxidation as compared to stress control (Sun et al. 2013; Shu et al. 2015). Noteworthy, the synergistic effect of PGR and PGPR was more pronounced.

The higher activities of antioxidant enzymes in the stress control is an indication of oxidative stress encountered by the crop (Almeselmani et al. 2006). The observed reduction of antioxidant enzymes by the PGR treatment may be attributed to the fact that PGPR reduced the occurrence of stress induced oxidative stress in plants subsequently the antioxidant enzymes were lesser in PGPR/PGR heated plants. SOD activity was higher in paddy due to salt stress but inoculation of PGPR decreased the SOD activity (Jha and Subramanian 2014). Khan et al. (2017) reported that the combined application of PGPR lead to significant decrease in CAT, POD and SOD activities in the leaves of chickpea grown under stress condition. Reduction in antioxidant enzymes activity by PGPR or PGR had been reported previously (Upadhyay et al. 2012; Mahsa Hosseini et al. 2015).

SA enhanced the leaf area alone and in combination with Put and PGPR. SA application was reported to increase leaf area in many crop plants grown under drought stress condition (Sadeghipour and Aghaei 2011; Fahraj et al. 2014). Gupta et al. (2012) reported 23% and 16% increases in leaf area of chickpea following exogenous applications of Put and benzyl adenine under water stress condition. Agbodjato et al. (2016) and Gholami et al. (2009) reported 45% and 91% enhancement in leaf area of maize under stress condition due to inoculation with *A. lipoferum*.

Promontory effects of PGPR and PGR have been reported on root length and root area. PGPR induced root growth may be attributed to production of Indole 3-Acetic Acid (IAA) and IAA had long been known for its stimulatory effects on root proliferation (Barazani and Friedman 1999). Erturk et al. (2010) reported 47% increase in rooting ratio when plants were inoculated with PGPR strains. Gamalerio et al. (2004) reported the promontory effects of *fluorescent pseudomonads* and an arbuscular mycorrhizal fungus on the growth and root parameters of tomato plant. The coinoculation of 3-PGPR had greater % increase on root length and root area. Foliar application of both SA and Put had significantly enhanced the root and shoot growth in many plants including soyabean, maize and chamomile (Khodary 2004; Rivas-San Vicente and Plasencia 2011).

**Conclusion**

It inferred that PGPR and PGR work synergistically to promote growth of plants under moisture and nutrient deficit condition of sandy soil. PGR are more responsive in the sensitive variety. Addition of PGR to PGPR inoculated plants assisted the plant in osmoregulation and amelioration of oxidative stresses and in induction of new proteins. Since, SA induces Systemic Acquired Resistance (SAR) in plants hence the addition of SA along with PGPR may render the plant more productive and tolerant to pathogen attack.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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