The extent of grain yield and plant growth enhancement by plant growth-promoting broad-spectrum Streptomyces sp. in chickpea

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Abstract

The physiological and molecular responses of five strains of Streptomyces sp. (CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27), with their proven potential for charcoal rot disease control in sorghum and plant growth-promotion (PGP) in sorghum and rice, were studied to understand the mechanisms causing the beneficial effects. In this investigation, those five strains were evaluated for their PGP capabilities in chickpea in the 2012–13 and 2013–14 post-rainy seasons. All of the Streptomyces sp. strains exhibited enhanced nodule number, nodule weight, root weight and shoot weight at 30 days after sowing (DAS) and pod number, pod weight, leaf area, leaf weight and stem weight at 60 DAS in both seasons over the un-inoculated control. At crop maturity, the Streptomyces strains had enhanced stover yield, grain yield, total dry matter and seed number plant\(^{-1}\) in both seasons over the un-inoculated control. In the rhizosphere, the Streptomyces sp. also significantly enhanced microbial biomass carbon, dehydrogenase activity, total nitrogen, available phosphorous and organic carbon in both seasons over the un-inoculated control. Of the five strains of Streptomyces sp., CAI-17, CAI-68 and CAI-78 were superior to KAI-26 and KAI-27 in terms of their effects on root and shoot development, nodule formation and crop productivity. Scanning electron microscopy (SEM) micrographs had revealed the success in colonization of the chickpea roots by all five strains. Quantitative real-time PCR (qRT-PCR) analysis of selected PGP genes of actinomycetes revealed the selective up-regulation of indole-3-acetic acid (IAA)-related and siderophore-related genes by CAI-68 and of \(\beta\)-1,3-glucanase genes by KAI-26.

Keywords: Field evaluation; Plant growth-promotion (PGP); qRT-PCR analysis; Scanning electron microscopy; Streptomyces sp.

Introduction

In recent years, the use of plant growth-promoting (PGP) bacteria has become one of the most attractive options for enhancing the sustainability of agricultural systems in many parts of the world due to their eco-friendliness, low production cost and reduced use of non-renewable resources. PGP bacteria promote plant growth through various mechanisms such as nitrogen fixation, the solubilization of phosphorus, the chelation of iron, the secretion of plant growth hormones and the inhibition of phytopathogens. Bacteria such as Bacillus sp., Pseudomonas sp. and Streptomyces sp. were found to play a major role both in mobilizing and acquiring nutrients and in controlling phytopathogens (Postma et al. 2003; Perner et al. 2006; Gopalakrishnan et al. 2011a, b, c; Jalilian et al. 2012). The presence of PGP bacteria in the rhizosphere has been shown to enhance shoot growth, root growth, root hair development, plant hormone concentrations, nitrogen fixation, the solubilization of minerals and the suppression of pathogens (Shaukat et al. 2006; Lucas et al. 2009; Richardson et al. 2009; Gopalakrishnan et al. 2012a, b). The PGP potential of Streptomyces sp. has been demonstrated on tomato, wheat, rice, bean and pea (Tokala et al. 2002; Nassar et al. 2003; El-Tarabily 2008; Sadeghi et al. 2012; Gopalakrishnan et al. 2013, 2014a). Streptomyces sp. are known to promote plant growth either by producing indole-3-acetic acid (IAA) and siderophores and/or by inhibiting soil-borne fungal pathogens (Aldesuquy et al. 1998; Trejo-Estrada...
et al. 1998; Tokala et al. 2002; Macagnan et al. 2008; Gopalakrishnan et al. 2011a, b, 2013; 2014a).

Previously, we have demonstrated the potential of a set of eight *Streptomyces* strains (CAI-21, CAI-26, MMA-32, CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27) isolated from herbal vermicompost, for the bio-control of charcoal rot disease, caused by *Macrophomina phaseolina* (Tassi) Goid., in sorghum (Gopalakrishnan et al. 2011b) and for PGP in rice (Gopalakrishnan et al. 2012a, 2013). The objectives of this investigation were to further evaluate five of the eight *Streptomyces* strains (CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27) for their PGP traits in chickpea under field conditions, to demonstrate gene expression profiles by qRT-PCR analysis, and to ensure colonizing ability in chickpea by scanning electron microscopy (SEM) analysis.

**Materials and methods**

*Streptomyces* strains

Five strains of *Streptomyces* spp., CAI-17 (GenBank accession number: JQ682619), CAI-68 (GenBank accession number: JQ682622), CAI-78 (GenBank accession number: JQ682623), KAI-26 (GenBank accession number: JQ682624) and KAI-27 (GenBank accession number: JQ682625), previously reported by us to have capacity for the bio-control of charcoal rot in sorghum (Gopalakrishnan et al. 2011b) and PGP in rice (Gopalakrishnan et al. 2013), were further studied in the present investigation.

**Evaluation of Streptomyces** sp. for PGP potential on chickpea under field conditions

This experiment was carried out during the 2012–13 and 2013–14 post-rainy cropping seasons at ICRISAT, Patancheru (17°30′N; 78°16′E; altitude 549 m) in peninsular India. Soils at the experimental site are classified as Vertisols (fine montmorillonitic isohyperthermic typic pallustert) having 52% clay, 21% silt and 26% sand, with an alkaline pH of 7.7–8.5 and an organic carbon content of 0.4–0.6%. The soil depth of the field used was ≥1.2 m, and this soil retained approximately 200 mm of plant-available water in a 120-cm (maximum rooting depth by chickpea) soil profile. The mineral content of the top 15 cm of the soil profile at the harvest stage and analysed for soil chemistry (total nitrogen [ppm], available phosphorus [ppm] and % organic carbon as per the protocols of Novozamsky et al. 1983, Olsen and Sommers 1982 and Nelson and Sommers 1982, respectively) and microbial biomass carbon (μg g⁻¹ soil) by the fumigation method and dehydrogenase activity (μg TPF g⁻¹ soil 24 h⁻¹) by the triphenyl formazan production method as per the protocols of Anderson and Domsch 1989 and Casida 1977, respectively.

**Colonization of Streptomyces** sp.

The roots of chickpea were examined for colonization by *Streptomyces* sp. by SEM (Bozzola and Russell 1998). For this procedure, the seeds of chickpea variety ICCV 2 were surface-sterilized first with 2.5% sodium hypochlorite solution (for five minutes), followed by 70% ethanol (for five minutes), and then rinsed with sterilized water (eight times) before being allowed to sprout in a Petri plate overnight. The sprouted seeds were transferred into test *Streptomyces* strains (CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27; grown in Bennett’s broth separately) for an hour before being sown in pots containing sterilized coarse sand (six seeds/8” plastic pot). The soil was drenched with a booster dose of *Streptomyces* strains (5 ml per seedling; 10⁸ CFU ml⁻¹) one week after sowing.
in sand. The pots were incubated for two weeks in a greenhouse at a temperature of 24 ± 2°C. At the end of the incubation period, chickpea seedlings were removed from the sand pots and the roots were washed in phosphate buffer (0.1 M; pH 7.2). The tip of the roots were cut into pieces 5 mm long and fixed in 2.5% glutaraldehyde in phosphate buffer for 24 h at 4°C. At the end of incubation, the samples were washed with phosphate buffer, postfixed in 2% osmium tetroxide for 4 h and dehydrated using a graded series of ethanol. The dehydrated samples were dried with critical-point liquid carbon dioxide as a transition fluid. The dried materials were adhered onto aluminium specimen mounts with double-stick adhesive tape. The samples were later coated with gold-palladium in an automated sputter coater (JEOL JFC-1600) and examined with a scanning electron microscope (JOEL-JSM 5600) as per the standardized protocols at RUSKA lab, College of Veterinary Science, Rajendranagar, Hyderabad, India. Observations of the presence of Streptomyces spores on root surfaces were recorded.

**Gene expression profile**

**RNA extraction**

The five selected Streptomyces sp. (CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27) were grown in Bennett’s broth at 28°C for 72 h. Total RNA was extracted from all of the Streptomyces strains using the “NucleoSpin RNA Plant” kit (Macherey-Nagel). The quality and quantity of RNA was estimated by a Nanodrop (Thermo Scientific) and RNA integrity was determined using a 2100 Bioanalyzer (Agilent).

**Quantitative real time- PCR (qRT-PCR)**

qRT-PCR was performed using the Applied Biosystems 7500 Real-Time PCR System with the SYBR green chemistry (Applied Biosystems, USA) as per the manufacturer’s instructions. Well-characterized genes Spaepen et al. 2007 relating to IAA production (F: GTACC CGG GATCTTCTTCAAC; R: GATGTCGGTTCTTGTC CAG) and siderophore (F: ATCCTCAACACCTGGTC TG; R: TCCTTGACTGTAGGAGTACTC) were collected from the UniprotKB database (http://www.uniprot.org/uniprot/) as described by Gopalakrishnan et al. (2014a). Similarly, genes for β-1,3-glucanase (F: CCG AACACCACCTACTCCAC; R: CCAGGTGAGGAT CAGGAAG) were also selected for the study. Gene-specific primers for real-time PCR were designed using Primer 3 software (Rosen and Skaletsky 2000). RNA polymerase principal sigma factor HrdB (SCOS5820) (F: GGTGAGGCTATCAACCAAGC; R: CTCGATGAGGT CACCGAACT) was used as the endogenous control. qRT-PCR reactions were performed as described earlier (Gopalakrishnan et al. 2014a). The data obtained were analysed using the mean of the CT values of the three biological replicates that were normalized to the mean CT values of the endogenous gene. The expression ratios were calculated using the 2^-ΔΔCt method. Relative transcription levels are presented graphically.

**Statistical analysis**

Analysis of variance) was performed by the GLM (General Linear Model) procedure of SAS software package (SAS Institute Inc 2013). Isolate were tested for significance and their means with control were compared by using Dunnett’s t-test.

**Results**

As all of the strains of Streptomyces spp. (CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27) enhanced different plant component performances, the means of all the five strains are presented. The Streptomyces-treated plots, by a mean of the five strains, enhanced agronomic performance in terms of nodule numbers (up to 61%), nodule weight (up to 44%) and shoot weight (up to 28%) at 30 DAS and plant height (up to 5%), pod numbers (up to 79%), pod weight (up to 89%), leaf area (up to 29%), leaf weight (up to 42%) and stem weight (up to 6%) at 60 DAS in both seasons (2012–13 and 2013–2014) over the un-inoculated control plots (Tables 1 and 2). At chickpea crop maturity, the Streptomyces-treated plots also enhanced stover yield (up to 41%), grain yield (up to 16%), total dry matter (up to 26%), pod weight (up to 7%), seed number (up to 21%) and seed weight (up to 11%) in both seasons over the un-inoculated control plots (Table 3). At crop maturity, in the top 15-cm of rhizosphere soil, the Streptomyces sp. treated plots enhanced the microbial biomass carbon (up to 59%) and dehydrogenase activity (up to 21%), as well as soil mineral nutrient contents, including total N (up to 8%), available P (up to 45%) and % organic carbon (up to 7%), in both seasons over the un-inoculated control plots (Tables 4 and 5).

SEM analysis of chickpea roots revealed a remarkable degree of colonization by Streptomyces sp. Roots from inoculated plants exhibited significant surface colonization by Streptomyces sp., while those from un-inoculated plants did not. The sporulation of Streptomyces sp. on the surface cell layer of chickpea roots was clearly evident for all five strains. The hyphae of Streptomyces sp. were also found to penetrate the surface cell layer of chickpea roots (Figure 1).

Gene expression profiles of plant-growth-promoting genes, IAA, siderophore and β-1,3-glucanase revealed overall up-regulation in the Streptomyces strains studied. The gene IAA showed higher up-regulation in the strain CAI-68 (13.7-fold), while no significant variation in the expression pattern was observed in the other four strains. The siderophore gene was highly up-regulated in strain CAI-68 (10.8-fold), followed by KAI-27 (3.6), and β-1,3-
glucanase expression was up-regulated in KAI-26 (9.3-fold) (Figure 2).

**Discussion**

The five strains of *Streptomyces* spp. (CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27), which are known for biocontrol of charcoal rot in sorghum and PGP in sorghum (Gopalakrishnan et al. 2011b) and rice (Gopalakrishnan et al. 2013), were evaluated for their PGP effects in chickpea under field conditions. In the present study, nodules were observed in the roots of chickpea on both the seasons (2012–13 and 2013–2014) though rhizobia were neither inoculated on seed nor applied on soil indicating the presence of native rhizobia in the soil. Rhizobia were always noted in the Vertisols of ICRISAT which depends on the presence of a sufficient population of bacteria, as well as the rhizosphere competence, root-feeding and root-harmonious bacteria, which lead to beneficial functions for crops such as plant growth-promotion, nitrogen fixation, phosphate solubilization and protection against pathogens. Although the *Streptomyces* spp. used in this study excrete antimicrobial compounds, antagonism tests by the poison food technique indicate that none of the strains inhibit the growth of nodulating bacteria *Rhizobium leguminosarum* on yeast extract mannitol agar media (data not shown). Thus, the *Streptomyces* spp., appears to be compatible with *Rhizobium*.

Colonization of chickpea roots by *Streptomyces* spp., at the right time and place is essential for enhanced PGP activity. Successful host-microbe interaction is essential, which depends on the presence of a sufficient population of bacteria, as well as the rhizosphere competence, root-

| Table 1 Response of various plant components of chickpea to the application of different strains of *Streptomyces* sp. at 30 days after sowing in a Vertisol during 2012–13 and 2013–14 post-rainy seasons | Year 2012–13 | Year 2013–14 |
|---|---|---|---|
| Isolate | Nodule number (plant⁻¹) | Nodule weight (mg plant⁻¹) | Root weight (mg plant⁻¹) | Shoot weight (g plant⁻¹) | Nodule number (plant⁻¹) | Nodule weight (mg plant⁻¹) | Root weight (mg plant⁻¹) | Shoot weight (g plant⁻¹) |
| CAI-17 | 20 | 36 | 186 | 2.11 | 51 | 228 | 192 | 1.76 |
| CAI-68 | 19 | 37 | 173 | 1.76 | 60 | 240 | 205 | 1.91 |
| CAI-78 | 21 | 71 | 184 | 1.81 | 59 | 242 | 207 | 1.75 |
| KAI-26 | 19 | 29 | 172 | 1.39 | 50 | 228 | 183 | 1.74 |
| KAI-27 | 19 | 36 | 172 | 1.55 | 50 | 224 | 186 | 1.74 |
| Control | 12 | 29 | 171 | 1.35 | 49 | 221 | 168 | 1.72 |
| Mean | 19 | 40 | 176 | 1.66 | 53 | 231 | 190 | 1.79 |
| SEM± | 1.04*** | 4.1*** | 3.3* | 0.06*** | 0.8*** | 4.2* | 5.75*** | 0.04* |
| CV% | 10 | 18 | 3 | 6 | 3 | 3 | 5 | 4 |

SEM = Standard Error of Mean; CV = Coefficients of variation; * = significant at p < 0.05, *** = significant at p < 0.001; According to Dunnett’s t-test, Isolate mean values are compared with control at p < 0.05.
| Isolate | Year 2012–13 | Year 2013–14 |
|---------|--------------|--------------|
|         | Plant height (cm) | Pod number (plant⁻¹) | Pod weight (g plant⁻¹) | Leaf area (cm² plant⁻¹) | Leaf weight (g plant⁻¹) | Stem weight (g plant⁻¹) | Plant height (cm) | Pod number (plant⁻¹) | Pod weight (g plant⁻¹) | Leaf area (cm² plant⁻¹) | Leaf weight (g plant⁻¹) | Stem weight (g plant⁻¹) |
| CAI-17  | 54*          | 65*          | 3.26*          | 1042*          | 7.25*          | 6.55*          | 49*          | 58*          | 4.18*          | 781*          | 4.45*          | 4.17*          |
| CAI-68  | 57*          | 72*          | 4.47*          | 868*          | 5.30*          | 4.87*          | 48*          | 66*          | 4.32*          | 667*          | 4.05*          | 4.75*          |
| CAI-78  | 51*          | 91*          | 3.41*          | 770*          | 6.39*          | 4.92*          | 50*          | 87*          | 5.58*          | 878*          | 4.67*          | 4.43*          |
| KAI-26  | 51*          | 72*          | 3.52*          | 906*          | 6.38*          | 5.18*          | 49*          | 58*          | 4.18*          | 832*          | 4.54*          | 4.17*          |
| KAI-27  | 48*          | 83*          | 4.50*          | 739*          | 5.56*          | 4.75*          | 47*          | 56*          | 4.49*          | 633*          | 4.00*          | 4.18*          |
| Control | 50           | 43           | 2.04           | 670           | 4.36           | 3.36           | 47           | 57           | 4.14           | 626           | 3.71           | 4.11           |
| Mean    | 52           | 71           | 3.53           | 833           | 5.87           | 4.94           | 48           | 64           | 4.48           | 736           | 4.24           | 4.30           |
| SE±     | 0.4***       | 1.9***       | 0.151***       | 31.0***       | 0.212***       | 0.071***       | 0.6*         | 3.3***       | 0.079***       | 15.1***       | 0.199*         | 0.096**        |
| CV%     | 1            | 5            | 7              | 6             | 6              | 3              | 2            | 9            | 3              | 4             | 8              | 4              |

SEM = Standard Error of Mean; CV = Coefficients of variation; * = significant at p < 0.05, ** = significant at p < 0.01, *** = significant at p < 0.001; According to Dunnett’s t-test, isolate mean values re compared with control at p < 0.05.
Table 3 Response of grain yield and its components to the application of different strains of *Streptomyces* sp. to chickpea in a Vertisol during 2012–13 and 2013–14 post-rainy seasons

| Isolate | Year 2012–13 | Year 2013–14 |
|---------|--------------|--------------|
|         | Stover yield (t ha\(^{-1}\)) | Grain yield (t ha\(^{-1}\)) | TDM (t ha\(^{-1}\)) | 1000-seed weight (g) | Seed number (plant\(^{-1}\)) | Seed weight (g plant\(^{-1}\)) | Stover yield (t ha\(^{-1}\)) | Grain yield (t ha\(^{-1}\)) | TDM (t ha\(^{-1}\)) | 1000-seed weight (g) | Seed number (plant\(^{-1}\)) | Seed weight (g plant\(^{-1}\)) |
| CAI-17  | 1.42\(^*\) | 2.01\(^*\) | 3.43\(^*\) | 220 | 22.2 | 72\(^*\) | 16.7 | 1.75 | 1.70\(^*\) | 3.45 | 196 | 180 | 72 | 15.0\(^*\) |
| CAI-68  | 1.31\(^*\) | 1.89\(^*\) | 3.20\(^*\) | 221 | 18.2 | 72\(^*\) | 15.4 | 2.03 | 1.70\(^*\) | 3.72\(^*\) | 198\(^*\) | 173 | 70 | 15.3\(^*\) |
| CAI-78  | 1.91\(^*\) | 2.16\(^*\) | 4.08\(^*\) | 227 | 18.2 | 55 | 14.1 | 2.19\(^*\) | 1.84\(^*\) | 4.03\(^*\) | 200\(^*\) | 210\(^*\) | 82 | 15.9\(^*\) |
| KAI-26  | 1.33\(^*\) | 1.96\(^*\) | 3.28\(^*\) | 231\(^*\) | 19.8 | 63\(^*\) | 14.6 | 1.69 | 1.76\(^*\) | 3.45 | 196 | 17.4 | 65\(^*\) | 14.3 |
| KAI-27  | 1.81\(^*\) | 1.81\(^*\) | 3.62\(^*\) | 222 | 17.2 | 63\(^*\) | 15.4 | 1.82 | 1.68\(^*\) | 3.51 | 196 | 17.5 | 67 | 13.6 |
| Control | 1.11 | 1.69 | 2.80 | 220 | 18.0 | 54 | 13.8 | 1.68 | 1.55 | 3.23 | 195 | 17.0 | 66 | 13.3 |
| Mean    | 1.48 | 1.92 | 3.40 | 223 | 18.9 | 63 | 15.0 | 1.86 | 1.71 | 3.57 | 197 | 18.0 | 70 | 14.6 |
| SEM±    | 0.020\(^***\) | 0.022\(^***\) | 0.034\(^***\) | 0.24\(^***\) | 0.5\(^***\) | 0.42\(^**\) | 0.093\(^*\) | 0.019\(^***\) | 0.098\(^**\) | 0.7\(^*\) | 0.23\(^***\) | 3.3\(^*\) | 0.30\(^***\) |
| CV%     | 2 | 2 | 2 | 1 | 2 | 2 | 5 | 9 | 2 | 5 | 1 | 2 | 8 | 4 |

TDM = total dry matter; SEM = Standard Error of Mean; CV% = Coefficients of variation; * = significant at p < 0.05, ** = significant at p < 0.01, *** = significant at p < 0.001; According to Dunnett’s t-test, isolate mean values are compared with control at p < 0.05.
colonizing ability and PGP capability of the bacteria (Lugtenberg and Dekkers 1999). The data for grain and stover yield, root mass and other agronomical traits, the chemical and biological activities of the rhizosphere soil along with the SEM micrograph clearly establish that the PGP effects of the *Streptomyces* spp., had been caused by successful colonization of the inoculated chickpea roots. The mechanism by which the five *Streptomyces* sp. could consistently enhance agronomical and yield traits on sorghum, rice (from our previous study) and chickpea can be attributed to their enzymatic activities, such as the ability to produce siderophores and indole acetic acid and β-1,3-glucanase (Gopalakrishnan et al. 2011b; 2013). Siderophores are known to form stable organic complexes with heavy metals such as Cu, Cd, In, U, Np, Al, Pb, Zn and Ga and increase the soluble metal concentration (Rajkumar et al. 2010) in the soil system, thus alleviating the various heavy metal stresses imposed on plants. Siderophores also act as solubilizing agents of iron from minerals when iron nutrition becomes a limitation (Indiragandhi et al. 2008). IAA-producing microorganisms are known to stimulate seed germination, initiate adventitious and lateral root formation and increase root length and surface area, thereby providing the host plant greater access to soil nutrients and water (Ahemad and Kibret 2014). The cell wall of plant pathogens is composed of layers of β-1,3-glucan (as in the case of *Fusarium oxysporum*, the causal agent of wilt disease in many crops) and lysis of these layers by β-1,3-glucanase-producing microorganisms leads to the leakage of cell contents and the collapse of the pathogenic fungi (Singh et al. 1999).

Our previous results documented the production of IAA (0.22 – 0.95%), β-1,3 glucanase (0.2 – 2.92 units) and siderophore (qualitative; 1–3 rating scale) by all of these *Streptomyces* strains (Gopalakrishnan et al. 2011b; Gopalakrishnan et al. 2013). Further characterization on gene expression profiles of these PGP genes revealed

### Table 4 Response of microbial biomass carbon and dehydrogenase activity in the chickpea rhizosphere to the application of different strains of *Streptomyces* spp. in a Vertisol during 2012–13 and 2013–14 post-rainy seasons

| Isolate | Microbial biomass C (μg g⁻¹ soil) | Dehydrogenase activity (μg TPF g⁻¹ soil 24 h⁻¹) | Microbial biomass C (μg g⁻¹ soil) | Dehydrogenase activity (μg TPF g⁻¹ soil 24 h⁻¹) |
|---------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| CAI-17  | 1221*                           | 52.7                            | 974*                            | 68.6                            |
| CAI-68  | 1295*                           | 56.8                            | 1000*                           | 71.5*                           |
| CAI-78  | 1424*                           | 63.6                            | 908*                            | 67.8                            |
| KAI-26  | 893*                            | 53.0                            | 842*                            | 688*                            |
| KAI-27  | 1132*                           | 56.8                            | 444                             | 59.0                            |
| Control | 752                             | 51.9                            | 550                             | 55.7                            |

| Mean    | 1119                            | 55.8                            | 786                             | 65.2                            |

SEM = Standard Error of Mean; CV = Coefficients of variation; * = significant at p < 0.05, ** = significant at p < 0.01, *** = significant at p < 0.001; According to Dunnett’s t-test, isolate mean values are compared with control at p < 0.05.

### Table 5 Response of rhizosphere soil chemical properties of chickpea at its maturity to the application of different strains of *Streptomyces* sp. in a Vertisol during the 2012–13 and 2013–14 post-rainy seasons

| Isolate | Total N (ppm) | Available P (ppm) | Organic carbon (%) | Total N (ppm) | Available P (ppm) | Organic carbon (%) |
|---------|---------------|------------------|-------------------|---------------|------------------|-------------------|
| CAI-17  | 656           | 11.2*            | 0.51*             | 737           | 11.1*            | 0.56              |
| CAI-68  | 703*          | 10.4             | 0.49*             | 744           | 10.2             | 0.54              |
| CAI-78  | 714*          | 12.2*            | 0.56*             | 815*          | 11.3*            | 0.67*             |
| KAI-26  | 666           | 10.8*            | 0.45              | 724           | 10.2             | 0.57              |
| KAI-27  | 668           | 10.8*            | 0.47              | 720           | 6.9              | 0.58              |
| Control | 632           | 10.1             | 0.47              | 735           | 6.9              | 0.55              |
| Mean    | 673           | 10.9             | 0.49              | 746           | 9.4              | 0.58              |

| SEMs   | 13.6*         | 0.13***          | 0.004***          | 15.2*         | 0.67**           | 0.016*            |
| CV%    | 3             | 2                | 1                 | 3             | 10               | 4                 |

SEM = Standard Error of Mean; CV = Coefficients of variation; * = significant at p < 0.05, ** = significant at p < 0.01, *** = significant at p < 0.001; According to Dunnett’s t-test, isolate mean values are compared with control at p < 0.05.
overall up-regulation in all the *Streptomyces* strains. However, no significant change in expression profiles of all three genes, IAA, siderophore and β-1,3-glucanase, was observed in isolates CAI-17 and CAI-78. The relative expression profiles of these genes in CAI-17 and CAI-78 do not correlate with the PGP capabilities observed under field conditions. This may be due to analysis of the gene expression profiles in only one biological replication of the strains. The exact role of these genes is to be precisely elucidated with more biological replications. However, validation of the IAA, siderophore and β-1,3-glucanase genes confirmed the insights gained from *in vitro* PGP attributes of the studied *Streptomyces* strains. Hence, it is concluded that the *Streptomyces* strains studied in this investigation contains multiple PGP traits and can therefore be exploited not only for PGP but also for biological control of plant pathogens.

The five *Streptomyces* spp. used in this study are apparently well adapted not only to the sorghum and rice rhizospheres, as reported earlier, but also to the chickpea rhizosphere, as demonstrated in the current investigation. Additionally, the five *Streptomyces* spp. contain a broad range of PGP abilities and demonstrate multiple beneficial actions. Therefore, the five *Streptomyces* spp. used in this study are potential candidates for the discovery of novel secondary metabolites and their usefulness in host plant resistance against a range of pathogens and
insect pests. These *Streptomyces* sp. strains can be useful in furthering the use of eco-friendly bio-pesticides and bio-fertilizers in integrated pest, disease and nutrition management programs. However, there is a need to determine the effectiveness of these *Streptomyces* strains under multi-location trials and to understand the nature of their interaction with other native soil micro-flora and micro-fauna with host plants and the environment.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

SG, VS, GA, BH, HK and RKV carried out the lab and field studies. AR done the statistical analysis and SG drafted the manuscript. All authors read and approved the final manuscript.

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