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# Evaluation of Broad-Spectrum *Streptomyces* sp. for Plant Growth Promotion Traits in Chickpea (*Cicer arietinum* L.)

**Subramaniam Gopalakrishnan*, Vadlamudi Srinivas, Gottumukkala Alekhya, Bandikinda Prakash, Himabindu Kudapa and Rajeev Kumar Varshney**

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Telangana, India
*Author for correspondence; e-mail: s.gopalakrishnan@cgiar.org; Phone: +91 40 3071 3610; Fax: +91 40 3071 3074

**The study evaluated three strains of *Streptomyces* (CAI-21, CAI-26 and MMA-32) for their plant growth promoting (PGP) traits in chickpea (*Cicer arietinum* L.) under field conditions in two consecutive post-rainy seasons, 2012–2013 and 2013–2014, to confirm colonizing ability in chickpea and to demonstrate gene expression profiles of indoleacetic acid, siderophore and β-1,3-glucanase genes. The *Streptomyces* enhanced nodule number, nodule weight, root weight and shoot weight at 30 d after sowing (DAS) and plant height, pod number, pod weight, leaf area, leaf weight and stem weight at 60 DAS in both seasons over the uninoculated control. At crop maturity, the *Streptomyces* enhanced stover yield, grain yield and total dry matter in both seasons over the uninoculated control. In the rhizosphere, the biological and mineral nutrient activities, such as microbial biomass carbon, dehydrogenase activity, total nitrogen, available phosphorus and organic carbon were also found higher in plots inoculated with *Streptomyces* in both seasons over the uninoculated control plots. Scanning electron microscopy analysis revealed that the *Streptomyces* colonized the roots of chickpea. Quantitative real time PCR analysis on selected PGP genes of *Streptomyces* revealed up-regulation of β-1,3-glucanase gene in CAI-26, β-1,3-glucanase and siderophore genes (siderophore synthetase) in CAI-21 and β-1,3-glucanase, siderophore (siderophore synthetase) and indole-3-acetic acid (IAAH and IAAM) genes in MMA-32. The three *Streptomyces* demonstrated broad-spectrum PGP activity.**

Key Words: plant growth promotion, *Streptomyces* sp., field demonstration, scanning electron microscopy, qRT-PCR analysis

Abbreviations: IAA – indoleacetic acid, PGP – plant growth promoting/promotion, qRT-PCR – quantitative real time polymerase chain reaction, SEM – scanning electron microscopy

## INTRODUCTION

In recent years, application of plant growth promoting (PGP) bacteria is gaining importance in sustainable agricultural systems because of their low production cost, consumption of less non-renewable resources and eco-friendliness in nature. The mechanisms involved in plant growth promotion by PGP bacteria include secretion of PGP hormones, chelation of iron, nitrogen fixation, solubilization of phosphorus and inhibition of plant pathogens (Tokala et al. 2002; Soares et al. 2006; Cheng et al. 2007; Hao et al. 2011; Panhwar et al. 2012). Inoculation of PGP bacteria on the rhizosphere had been reported to enhance root and shoot growth, nitrogen fixation and solubilization of minerals (Shaukat et al. 2006; Richardson et al. 2009). Strains of *Pseudomonas*, *Bacillus* and *Streptomyces* were reported to help the plants not only by mobilizing the nutrients but also by controlling plant pathogens (Postma et al. 2003; Perner et al. 2006; Gopalakrishnan et al. 2011a, b).

*Streptomyces,* Gram-positive bacteria with high G + C content, are abundantly found in the rhizosphere of agriculturally important crops and compost. They are known for their PGP traits, the breakdown of carbohydrates such as chitin and cellulose and for degradation of soils contaminated with pesticides and heavy metals. PGP potential of *Streptomyces* was reported on cereals such as wheat (Sadeghi et al. 2012) and rice (Gopalakrishnan et al. 2013, 2014), legumes such as bean (Nassar et al. 2003) and pea (Tokala et al. 2002) and vegetables such as tomato (El-Tarabily 2008). *Streptomyces* promote plant growth by producing indole-3-acetic acid (IAA) (Aldesuquy et al. 1998) or siderophores (Tokala et al. 2002). Besides, *Streptomyces* have been extensively used for biocontrol of soil-borne fungal pathogens (Mahadevan and Crawford 1997; Trejo-Estrada et al. 1998; Macagnan et al. 2008; Gopalakrishnan et al. 2011b).

Chickpea (*Cicer arietinum* L.) is the third most important food legume crop in the world, after bean and soybean, with a total production of 11.6 million tons from an area of 13.2 million ha and a productivity of 880 kg $ha^{-1}$ (FAOSTAT 2011). Global yields of chickpea have been relatively stagnant for the last two decades in spite of the use of various conventional and molecular breeding approaches. Hence, in the present study, we proposed the use of PGP *Streptomyces* as a tool to enhance the plant growth and yield of chickpea. Previously, we demonstrated a set of three *Streptomyces* strains (CAI-21, CAI-26 and MMA-32) isolated from herbal vermicompost, with the potential for bio-control of charcoal rot disease, caused by *Macrophomina phaseolina* (Tassi) Goid., in sorghum (Gopalakrishnan et al. 2011a) and for PGP in rice (Gopalakrishnan et al. 2012). The objectives of this investigation were to further demonstrate the three *Streptomyces* strains for their PGP traits in chickpea under field conditions, to confirm colonizing ability in chickpea by scanning electron microscopy (SEM) analysis and to demonstrate gene expression profiling studies by quantitative real time PCR (qRT-PCR) analysis.

## MATERIALS AND METHODS

### *Streptomyces* Strains

Three strains of *Streptomyces*, CAI-21 (NCBI accession: JQ682620), CAI-26 (NCBI accession: JQ682621) and MMA-32 (NCBI accession: JQ682626) reported earlier by us as having potential for bio-control of charcoal rot in sorghum caused by *Macrophomina phaseolina* (Gopalakrishnan et al. 2011a) and PGP in rice (Gopalakrishnan et al. 2012), were further studied.

The experiment was done for two consecutive post-rainy cropping seasons in 2012−13 and 2013–14 at the International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru (17°30' N; 78°16' E; altitude 549 m) in Telangana State of India. Rhizosphere soils (0–15 cm) at the experimental field are classified as Vertisols (fine montmorillonitic isohyperthermic typicpallustert) having 53% clay, 21% silt and 25% sand with an alkaline pH of 7.6−8.3 and an organic carbon content of 0.4−0.6%.

The mineral content of the rhizosphere soil include 24.7, 8.6 and 298 mg $kg^{-1}$ soil of available nitrogen, available phosphorus and available potassium, respectively. The soil depth of the experimental site was ≥ 1.2 m and this soil retained 200 mm of plant-available water. The experimental field was kept fallow except for the post-rainy season crop. The field was prepared into broad beds and furrows with 1.2-m wide beds flanked by 0.3-m furrows in both seasons (2012−2013 and 2013–2014).

Surface application and incorporation of 18 and 20 kg of N and P $ha^{-1}$, respectively, as di-ammonium phosphate (DAP), were applied 3 d before sowing in both seasons. The experiment was laid out with three replicates and subplot sizes of 4 m × 3 ridges in a randomized complete block design (RCBD).

### Evaluation of *Streptomyces* Strains on Chickpea under Field Conditions

The test *Streptomyces* (CAI-21, CAI-26 and MMA-32) were grown individually on a starch casein broth at 28 °C for 4–5 d. Seeds of chickpea (variety ICCV2) were treated individually with a *Streptomyces* strain ($10^8$ CFU $ml^{-1}$) for 45 min and sown by hand planting on 10 October 2012 in the 1st year and on 2 November 2013 in the 2nd year in rows 30 cm apart at a depth of 5 cm to have an estimated plant stand of at least 26 plants $m^{-2}$. *Streptomyces* strain (1000 mL; $10^8$ CFU $mL^{-1}$) was applied once in 15 d, from the day of sowing, on the soil close to the plant until the flowering stage. Control plots contained no strains of *Streptomyces*.

Weeding was performed as and when required while the plots were irrigated at 21 and 49 d after sowing. No serious plant pathogens or insect pest attacks were observed during the cropping period. The crop was harvested manually on 17 January 2013 in the 1st year and on 7 Feb 2014 in the 2nd year.

Samplings were done at 30 d after sowing (DAS), 60 DAS and at crop maturity. On the day of sampling, plant aerial and underground parts were harvested separately with care to eliminate border effects in each plot, and dried to constant weight in dryers equipped with high air draught at 45 °C for 72 h; total dry weights were recorded.

At 30 DAS, nodule number, nodule weight, root weight and shoot weight and at 60 DAS, plant height, pod number, pod weight, leaf area, leaf weight and stem weight were recorded in both seasons (2012–2013 and 2013–2014). At crop maturity, stover yield, grain yield, total dry matter (TDM), 1000-seed weight, pod weight, seed number and seed weight were recorded in both seasons.

Rhizosphere soil samples were collected from 0–15 cm soil profile at crop maturity and analyzed for soil mineral nutrients (total nitrogen, available

phosphorus and organic carbon per the protocols of Novozamsky et al. 1983, Olsen and Sommers 1982 and Nelson and Sommers 1982, respectively) and soil biological activity (microbial biomass carbon by the fumigation method and dehydrogenase activity by the triphenyl formazan production method per the protocols of Anderson and Domsch 1989 and Casida 1977, respectively).

**Colonization Studies**

The colonizing capability of *Streptomyces* strains on the roots of chickpea was examined by scanning electron microscopy (SEM) analysis per the protocols of Bozzola and Russell (1998). In brief, the seeds of chickpea (variety ICCV2) were surface-sterilized at first with 2.5% sodium hypochlorite, followed by 70% ethanol (for 5 min each) and rinsed thoroughly with sterilized water before being allowed to sprout in a Petri dish overnight. The sprouted seeds were soaked into test *Streptomyces* strains (CAI-21, CAI-26 and MMA-32; grown in Bennett's broth separately) for 45 min before being sown in pots containing sterilized coarse sand (six seeds/8″ plastic pot).

Booster doses of *Streptomyces* strains ($10^8$ CFU $mL^{-1}$; 5 mL per seedling) were applied upon germination of the seed by soil drench method. The pots were incubated at 24 ± 2 °C in a greenhouse for 2 wk. At the end of the 2-wk incubation period, seedlings of chickpea were removed carefully from the pots and the roots were washed in 0.1 M phosphate buffer (pH 7.2). The tip of the roots were cut into 4–5 mm long pieces and fixed in glutaraldehyde (2.5%) in phosphate buffer for 24 h at 4 °C. At the end of 24 h incubation period, the root samples were again washed with phosphate buffer, post-fixed in osmium tetroxide (2%) 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 and adhered onto aluminum specimen mounts with double-stick adhesive tape. The mounted root samples were coated with gold-palladium in an automated sputter coater (JEOL JFC-1600) and examined under a scanning electron microscope (JOEL-JSM 5600) per the standardized protocols at RUSKA lab, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India.

**Gene Expression Profiling**

The three selected *Streptomyces* strains (CAI-21, CAI-26 and MMA-32) were grown in Bennett's broth at 28 °C for 72 h. At the end of the incubation period, total RNA was extracted from all the *Streptomyces* per the conventional Trizol method. The quality and quantity of RNA was estimated by a Nanodrop (Thermo Scientific, USA) and RNA integrity was estimated by a 2100 Bioanalyzer (Agilent, USA).

Quantitative real time PCR (qRT-PCR) was performed using the Applied Biosystems 7500 Real Time PCR System with the SYBR green chemistry (Applied Biosystems, USA) per the manufacturer's protocols. Genes relating to IAA (F: GTCACC GGGATCTTCTTCAAC; R: GATGTCGGTGTTC TTGTCCAG; IAAH and IAAM)) and siderophore (F: ATCCTCAACACCCTGGTCTG; R: TCCTT GTACTGGTACGGGACTT; siderophore synthetase) production were collected from the UniprotKB database (http://www.uniprot.org/uniprot) as described by Gopalakrishnan et al. (2014).

Similarly, genes for β-1,3-glucanase (F: CCGAACACCACCTACTCCAC; R: CCAGGTT GAGGATCAGGAAG) were also selected for the study. IAA (IAAH and IAAM), siderophore and β-1,3-glucanase gene-specific primers for qRT-PCR were designed using Primer3 software (Rosen and Skaletsky 2000). RNA polymerase principal sigma factor *HrdB* (SCO5820) (F: GGTCGAGGTCAT CAACAAGC; R: CTCGATGAGGTCACCGA ACT) was used as the endogenous control. qRT-PCR reactions and conditions were conducted as described earlier (Gopalakrishnan et al. 2014).

In brief, PCR reactions were carried out in 10 µL reactions containing 30 ng of first strand cDNA, 1X PCR buffer, 125 mMdNTPs, 1.5 mM MgCl2, 0.2 mM primers and 1U Taq polymerase and programmed as follows: 50 °C for 2 min and denaturation at 95 ºC for 10 min followed by 40 cycles of denaturation at 95 ºC for 15 sec and annealing and extension at 60 ºC for 1 min. The data obtained from different PCR runs or cDNA samples were analyzed 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 $2^{-\Delta\Delta Ct}$ method and relative transcription levels are presented graphically.

**Statistical Analysis**

Data were analyzed by analysis of variance (ANOVA) and the GLM (General Linear Model) procedure in the software package SAS (SAS Inst. 2002-08, SAS V9.3) considering isolates and replication as fixed in Randomized Complete Block Design (RCBD). Isolate means were tested for significance and compared using Fisher's protected least significant difference (LSD) test.

## RESULTS

### Evaluation of *Streptomyces* Strains on Chickpea under Field Conditions

The plots treated with *Streptomyces* strains CAI-21, CAI-26 and MMA-32 showed significantly enhanced agronomic performance of all the traits measured at 30 and 60 DAS. As all the strains enhanced different plant component performances, the means of all the three strains are presented. As seen at 30 DAS, by a mean of the three strains, the nodule numbers were enhanced by 71% in 2012–13 and 19% in 2013–14, nodule weight by 95% and 19%, root weight by 9% and 14% and shoot weight by 27% in 2012–13 and 35% in 2013–14 over the uninoculated control (Table 1).

Similarly at 60 DAS, the plant height was enhanced by 4% in 2012–13 and 5% in 2013–14, pod numbers by 97% and 35%, pod weight by 181% and 36%, leaf area by 13% and 25%, leaf weight by 26% and 23% and stem weight by 41% in 2012–13 and 17% in 2013–14 over the uninoculated control (Table 2). At crop maturity, the *Streptomyces* strains enhanced the stover yield by 37% in 2012–13 and 9% in 2013–14, grain yield by 13% and 12%, total dry matter by 22% and 10%, pod weight by 2% and 18%, seed number by 3% and 13% and seed weight by 2% in 2012–13 and 13% in 2013–14 over the uninoculated control plots (Table 3).

At crop maturity, in the top 15-cm rhizosphere soils, the *Streptomyces* strains-treated plots enhanced the soil biological activities including the microbial biomass carbon by 57% in 2012–13 and 56% in 2013–14 and dehydrogenase activity by 13% and 32% as well as soil mineral nutrient contents including total N by 11% and 4%, available P by 14% and 21% and organic carbon by 7% in 2012–13 and 6% in 2013–14 over the uninoculated control plots (Tables 4 and 5).

### Colonization Studies

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

### Gene Expression Studies

In the PGP gene expression profile studies, good quality RNA was isolated from all the three *Streptomyces* strains. qRT-PCR analysis on the three selected PGP genes of *Streptomyces* strains revealed

**Table 1.** Effect of the three *Streptomyces* sp. on agronomic performance of chickpea under field conditions at 30 d after sowing.

| Isolate | Year 2012-13 | | | | Year 2013-14 | | | |
|---|---|---|---|---|---|---|---|---|
| | 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-21 | 22 | 56 | 201 | 2.16 | 69 | 265 | 209 | 2.69 |
| CAI-26 | 21 | 54 | 174 | 1.52 | 49 | 236 | 196 | 2.09 |
| MMA-32 | 21 | 60 | 187 | 1.47 | 57 | 288 | 173 | 2.17 |
| Control | 12 | 29 | 171 | 1.35 | 49 | 221 | 168 | 1.72 |
| Mean | 19 | 50 | 183 | 1.62 | 56 | 253 | 187 | 2.17 |
| SE± | 0.7*** | 1.6*** | 2.1*** | 0.043*** | 0.8*** | 9.9** | 5.3** | 0.122** |
| LSD (5%) | 2.3 | 5.4 | 7.3 | 0.149 | 2.7 | 34.3 | 18.3 | 0.422 |
| CV% | 5 | 6 | 2 | 5 | 5 | 7 | 5 | 10 |

SE= Standard error; LSD= Least significant difference; CV= Coefficient of variation; **= Statistically significant at 0.01, ***= Statistically significant at 0.001

**Table 2.** Effect of the three *Streptomyces* sp. on agronomic performance of chickpea under field conditions at 60 d after sowing.

| Isolate | Year 2012-13 | | | | | | Year 2013-14 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Plant height (cm) | Pod number (plant$^{-1}$) | Pod weight (g plant$^{-1}$) | Leaf area (cm$^{-2}$ 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 (m$^{-2}$ plant$^{-1}$) | Leaf weight (g plant$^{-1}$) | Stem weight (g plant$^{-1}$) |
| CAI-21 | 54 | 85 | 4.56 | 793 | 5.21 | 4.92 | 48 | 83 | 5.63 | 845 | 4.53 | 4.89 |
| CAI-26 | 51 | 86 | 6.41 | 773 | 6.03 | 4.85 | 49 | 65 | 4.96 | 680 | 4.09 | 4.26 |
| MMA-32 | 50 | 82 | 6.25 | 700 | 5.22 | 4.48 | 51 | 90 | 6.65 | 845 | 5.44 | 5.66 |
| Control | 50 | 43 | 2.04 | 670 | 4.36 | 3.36 | 47 | 59 | 4.21 | 632 | 3.71 | 4.21 |
| Mean | 51 | 74 | 4.82 | 734 | 5.21 | 4.40 | 49 | 74 | 5.36 | 751 | 4.44 | 4.76 |
| SE± | 0.6** | 3.5*** | 0.254*** | 22.3* | 0.202** | 0.074*** | 0.8* | 1.3*** | 0.063*** | 23.4*** | 0.248** | 0.208** |
| LSD (5%) | 2.1 | 12.3 | 0.879 | 77.1 | 0.699 | 0.257 | 2.8 | 4.5 | 0.216 | 81.0 | 0.859 | 0.721 |
| CV% | 2 | 8 | 9 | 5 | 7 | 3 | 3 | 3 | 2 | 5 | 10 | 8 |

SE= Standard error; LSD= Least significant difference; CV= Coefficient of variation; *= Statistically significant at 0.05; **= Statistically significant at 0.01; ***= Statistically significant at 0.001

**Table 3.** Effect of the three *Streptomyces* sp. on agronomic performance and yield potential of chickpea under field conditions at harvest.

| 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) | Pod weight (g plant$^{-1}$) | 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) | Pod weight (g plant$^{-1}$) | Seed number (plant$^{-1}$) | Seed weight (g plant$^{-1}$) |
| CAI-21 | 1.33 | 1.85 | 3.18 | 221 | 19.0 | 55.3 | 13.1 | 1.71 | 1.86 | 3.57 | 196 | 17.1 | 67 | 13.5 |
| CAI-26 | 1.50 | 1.92 | 3.42 | 221 | 18.1 | 54.7 | 12.7 | 2.08 | 1.86 | 3.94 | 201 | 22.2 | 82 | 16.3 |
| MMA-32 | 1.71 | 1.96 | 3.67 | 224 | 18.0 | 55.0 | 13.1 | 1.69 | 1.87 | 3.35 | 200 | 20.9 | 76 | 15.4 |
| Control | 1.11 | 1.69 | 2.79 | 220 | 17.9 | 53.7 | 12.7 | 1.68 | 1.66 | 3.34 | 195 | 17.0 | 66 | 13.3 |
| Mean | 1.41 | 1.85 | 3.26 | 222 | 18.2 | 54.7 | 12.9 | 1.79 | 1.81 | 3.55 | 198 | 19.3 | 73 | 14.6 |
| SE± | 0.057*** | 0.021*** | 0.055*** | 0.2*** | 0.10** | 0.32* | 0.08* | 0.045** | 0.013*** | 0.047*** | 0.5*** | 1.07* | 2.4** | 0.51** |
| LSD (5%) | 0.196 | 0.072 | 0.19 | 0.9 | 0.38 | 1.10 | 0.27 | 0.155 | 0.046 | 0.164 | 1.6 | 3.71 | 8.30 | 1.78 |
| CV% | 7 | 2 | 3 | 1 | 1 | 1 | 1 | 4 | 1 | 2 | 1 | 10 | 6 | 6 |

TDM = Total dry matter; SE= Standard error; LSD= Least significant difference; CV= Coefficient of variation;
* = Statistically significant at 0.05; ** = Statistically significant at 0.01; *** = Statistically significant at 0.001

**Table 4.** Effect of the three *Streptomyces* sp. on rhizosphere soil biological activity in chickpea under field conditions at harvest.

| Isolate | Year 2012-13 | | Year 2013-14 | |
|---|---|---|---|---|
| | 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-21 | 1238 | 59.5 | 880 | 64.1 |
| CAI-26 | 1254 | 68.2 | 872 | 85.8 |
| MMA-32 | 1058 | 60.0 | 820 | 70.8 |
| Control | 752 | 55.4 | 550 | 55.7 |
| Mean | 1076 | 60.8 | 781 | 69.1 |
| SE± | 31.0** | 1.58* | 32.5** | 3.19* |
| LSD (5%) | 139.4 | 7.11 | 146.1 | 14.37 |
| CV% | 4 | 4 | 6 | 7 |

SE= Standard error; LSD= Least significant difference; CV= Coefficient of variation;
*= Statistically significant at 0.05; ** = Statistically significant at 0.01

**Table 5.** Effect of the three *Streptomyces* sp. on rhizosphere soil chemical properties in chickpea under field conditions at harvest.

| Isolate | Year 2012-13 | | | Year 2013-14 | | |
|---|---|---|---|---|---|---|
| | Total N (ppm) | Available P (ppm) | Organic Carbon % | Total N (ppm) | Available P (ppm) | Organic Carbon % |
| CAI-21 | 690 | 12.0 | 0.48 | 738 | 10.0 | 0.58 |
| CAI-26 | 761 | 11.5 | 0.53 | 796 | 7.7 | 0.61 |
| MMA-32 | 650 | 11.0 | 0.48 | 728 | 7.3 | 0.57 |
| Control | 632 | 10.1 | 0.47 | 725 | 6.9 | 0.55 |
| Mean | 683 | 11.1 | 0.49 | 747 | 8.0 | 0.57 |
| SE± | 6.2** | 0.096** | 0.007* | 9.6* | 0.30* | 0.008* |
| LSD (5%) | 27.8 | 0.431 | 0.032 | 43.0 | 1.36 | 0.034 |
| CV% | 1 | 1 | 2 | 2 | 5 | 2 |

*= Statistically significant at 0.05; **= Statistically significant at 0.01; SE= Standard error; LSD= Least significant difference; CV= Coefficient of variation

up-regulation of β-1,3-glucanase and siderophore (siderophore synthetase) genes in CAI-21 by 1.56 and 1.98-folds, respectively. The gene IAA (IAAH and IAAM) did not show any significant difference in the CAI-21. In the strain CAI-26, β-1,3-glucanase showed up-regulation by 1.27-fold; the genes siderophore (siderophore synthetase) and IAA (IAAH and IAAM) did not show any significant differences. Interestingly all the three genes showed up-regulation in MMA-32. The gene β-1,3-glucanase was up-regulated by 4.8-fold, siderophore by 12.2-fold and IAA by 7.7-fold in MMA-32 (Fig. 2).

## DISCUSSION

The three *Streptomyces* strains (CAI-21, CAI-26 and MMA-32) studied in this investigation were reported previously to have potential for control of charcoal rot in sorghum and PGP in sorghum (Gopalakrishnan et al. 2011a) and rice (Gopalakrishnan et al. 2012). In the present investigation, when these strains were evaluated for their PGP potential in chickpea under field conditions, all the three strains of *Streptomyces* enhanced agronomic and yield parameters in chickpea including root and shoot development, nodule formation and crop productivity over the uninoculated control in two consecutive post-rainy

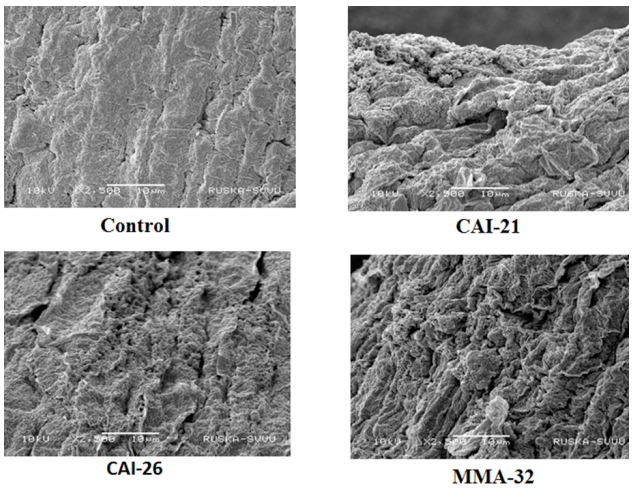


**Fig. 1.** Scanning electron microscopy photographs of the three *Streptomyces* sp. showing colonization on the roots of chickpea. Arrows indicate roots colonized by plant-growth promoting (PGP) *Streptomyces* sp.

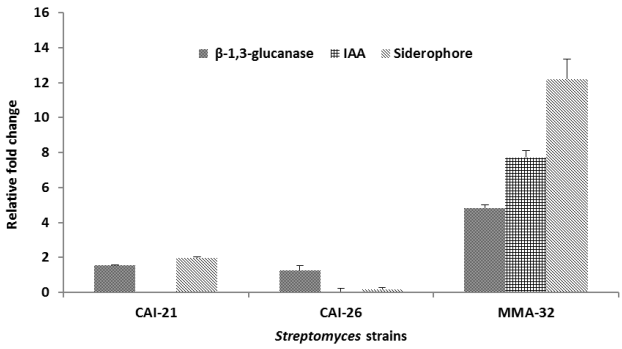


**Fig. 2.** Expression profile of plant-growth promoting (PGP) genes of *Streptomyces* strains.

seasons, 2012–13 and 2013–14. Also, these strains were found to enhance soil biological and mineral nutrient activities in the rhizosphere in both seasons over the uninoculated control. It was not possible to single out the best strain among the three *Streptomyces* as all the three strains were found superior in terms of root and shoot development, nodule formation, crop productivity and soil biological and mineral nutrient activities. Further, the three *Streptomyces* did not inhibit the growth of *Mesorhizobium ciceri* on yeast extract mannitol agar media (data not shown). Thus, it is concluded that the three *Streptomyces* strains are capable of enhancing agronomic and yield traits in chickpea and are compatible with *Rhizobium*, so that co-inoculation of these two genera is possible.

The efficacy of *Streptomyces* for PGP in agriculturally important crops, including cereals and legumes, was reported widely (Tokala et al. 2002; Nassar et al. 2003; El-Tarabily 2008; Sadeghi et al. 2012; Gopalakrishnan et al. 2014). PGP bio-agents with broad spectrum properties offer effective novel strategies not only for crop growth and yield but also for controlling insect pests and pathogens that attack crops. PGP bio-agents also bring forth induced systemic resistance against a broad range of pathogens and insect pests (Jetiyanon and Kloepper 2002; Ryu et al. 2007).

Host-microbe interaction is essential for colonization which depends on sufficient population of bacteria, rhizosphere competence of bacteria and roots colonizing and PGP ability of the bacteria (Lugtenberg and Dekkers 1999). Also, colonization by PGP bacteria at the right time and place is prerequisite for enhanced PGP activity. In the present investigation, SEM analysis demonstrated colonization of *Streptomyces* strains on the roots of chickpea. The SEM studies were done under greenhouse and sterilized conditions with pure cultures of *Streptomyces* strains. Hence, the colonized organism in the roots is of our strain only. Hence, the SEM analysis in addition to the data for grain and stover yield, root and other agronomical traits, the mineral nutrient and biological activities of the rhizosphere soil strongly suggest that the three *Streptomyces* strains had multiplied and colonized on the chickpea roots.

The mechanism by which the three *Streptomyces* strains consistently enhanced agronomical and yield traits on sorghum and rice (from our previous study) and chickpea (from this study) could be attributed to their ability to produce IAA, siderophores and β-1,3-glucanase activities (Gopalakrishnan et al. 2011a; 2012). IAA is the plant hormone that accelerates plant growth by enhancing shoot/root growth and seedling vigor. IAA-producing bacteria are known to stimulate seed germination, initiate root formation and increase root length and surface area thereby provides the host plant greater access to water and soil nutrients (Ahemad and Kibret 2014). Bacteria are known to have the ability to synthesize low molecular weight siderophores that are capable of sequestering $Fe^{3+}$. Siderophores act as solubilizing agents of iron from minerals under conditions of iron limitation (Indiragandhi et al. 2008). Siderophores form stable complexes with heavy metals such as Al, Cd, Cu, Ga, In, Np, Pb, U and Zn and increase the soluble metal concentration (Rajkumar et al. 2010). Hence, siderophores help to alleviate the stresses imposed on plants by heavy metals in soils.

The cell wall of phytopathogens, such as *Fusarium oxysporum* (causes wilt in many crops), contains layers of β-1,3-glucan and lysis of these layers by β-1,3-glucanase-producing bacteria leads

to the leakage of cell contents and the collapse of the pathogenic fungi (Singh et al. 1999). Hence, it is concluded that the *Streptomyces* strains used in this investigation apparently contained a broad range of PGP traits which can be exploited not only for PGP but also for biological control of plant pathogens.

Furthermore, qRT-PCR validation of PGP genes revealed that IAA gene showed higher up-regulation only in MMA-32 (7.7-fold), siderophore gene in MMA-32 (12.2-fold) followed by CAI-21 (1.98-fold) and β-1,3-glucanase gene in MMA-32 (4.8-fold) followed by CAI-21 (1.56-fold) and CAI-26 (1.27-fold), in descending order. The *Streptomyces* strain MMA-32 produced higher level of IAA and siderophores compared with the other two strains in the *in-vitro* PGP traits studies (Gopalakrishnan et al. 2011a) and expression profiling of the genes IAA and siderophore confirmed these results.

## CONCLUSION

The three *Streptomyces* strains studied in this investigation were apparently well adapted not only in sorghum and rice rhizosphere, as reported earlier, but also in the chickpea rhizosphere. Further, the strains apparently contained a broad range of PGP traits including IAA, siderophore and β-1,3-glucanase, indicating their broad spectrum activity. Hence, the three strains 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 insect pests and pathogens can help in furthering the use of eco-friendly biopesticides and biofertilizers. However, there is a need to do additional comprehensive research to exploit the potential of these PGP *Streptomyces* under different field conditions (multi-location trials) for commercialization and to improve sustainability in agricultural production.

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