

## Full Length Research Paper

# Plant growth-promotion by *Streptomyces* spp. in sorghum (*Sorghum bicolor* L.)

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Seven strains of *Streptomyces* spp.: BCA-546 (KF770898), BCA-659 (KF770889), BCA-667 (KF770888), BCA-689 (KF770899), BCA-698 (KF770900), CAI-133 (KF770895) and CAI-8 (KF770890), reported earlier to produce biocontrol and plant growth-promoting (PGP) substances were further evaluated for PGP traits in sorghum under greenhouse and field conditions. Under greenhouse conditions, plant height, leaf area and weight, root length and weight, shoot weight, panicle weight and seed weight were enhanced in plots inoculated with *Streptomyces* spp. than the un-inoculated control at 30, 60 days after sowing (DAS) and at final harvest. Similarly, treatment with *Streptomyces* spp. led to growth and yield enhancements under field conditions at 60 DAS and final harvest. Among the seven strains, BCA-698, BCA-689, BCA-546 and BCA-659 were found to be superior for PGP. Under field conditions, at both flowering and harvest stages, the soil organic C, available P and total N were also found to improve with *Streptomyces* spp. treatments. A scanning electron microscopic study showed extensive root colonization of sorghum. The gene expression profiles revealed up-regulation of  $\beta$ -1,3-glucanase, indole acetic acid (IAA) and siderophore genes. Based on the present findings, the seven selected *Streptomyces* strains could be employed to enhance plant growth and yield in sorghum.

**Key words:** Gene expression, plant growth-promotion, scanning electron microscopy, sorghum, *Streptomyces* spp.

## INTRODUCTION

Sorghum (*Sorghum bicolor* L.) has been an important staple food in semi-arid tropics of Asia and Africa for centuries. It is the fifth most important cereal crop in the world. Sorghum is widely used as food, for production of alcoholic beverages, bio-fuel, starch, adhesives and paper. Lower yield in sorghum may be attributed to biotic and abiotic stresses in addition to lower yield potential of

local landraces, poor agronomic practices, and low nutrient uptake and soil fertility.

Microorganisms can be beneficial to plant either by increasing the availability of both macro- and micro-elements such as nitrogen, phosphorus, iron and zinc in the rhizosphere (Cakmakci et al., 2006) or by producing plant growth-promoting (PGP) substances such as indole

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acetic acid (IAA) and siderophore (Vivas et al., 2006; Hanane et al., 2008). Soil microorganisms not only have the capability to produce compounds that are potentially promoting plant growth and yield but also inhibit phytopathogens by producing phthoxazolins, phosphinothricin and gougerotin (Murao and Hideo, 1983; Shiomi et al., 1995). Among the soil microorganisms, bacteria and fungi have received considerable attention as plant growth-promoters and biocontrol agents. For instance, plant growth-promoting *Pseudomonas chlororaphis* SRB 127, *Penicillium citrinum* VFI-51 and *Bacillus* spp. (Das et al., 2008; Haiyambo et al., 2015; Sreevidya and Gopalakrishnan, 2016) were shown to have antagonistic potential against *Macrophomina phaseolina*, a charcoal rot pathogen and other pathogens of sorghum.

Actinomycetes are important producers of bioactive compounds such as chitinase,  $\beta$ -1,3-glucanase and various antifungal substances (Rothrock and Gottlieb, 1984; Xiao et al., 2002; El-Tarabily and Sivasithamparam, 2006). Actinomycetes also produce extracellular active compounds such as IAA, phosphate solubilizing substances and intracellular siderophores, which induce germination of seeds and their growth (Hong et al., 2000; Zhang et al., 2000; Venkatachalam et al., 2010). Within actinomycetes, *Streptomyces* spp. have been investigated predominantly, mainly because of their dominance and the ease of isolation and their ample capacity for production of secondary metabolites, such as antibiotics and extracellular enzymes (El-Tarabily et al., 2000; Inbar et al., 2005; Carla et al., 2008; Sreevidya et al., 2015). Some of the *Streptomyces* sp. were also reported to have both PGP and antagonistic potentials against charcoal rot disease in sorghum (Ding et al., 2004; Gopalakrishnan et al., 2013a). Seven *Streptomyces* spp. (BCA-546, BCA-659, BCA-667, BCA-689, BCA-698, CAI-8 and CAI-133) were earlier reported to have PGP and biocontrol traits in chickpea (Alekhya and Gopalakrishnan, 2016). In the present investigation, the seven *Streptomyces* spp. were evaluated further for their PGP and yield enhancement potentialities in sorghum.

## MATERIALS AND METHODS

### PGP microbes

Seven strains of *Streptomyces* spp.: BCA-546 (KF770898), BCA-659 (KF770889), BCA-667 (KF770888), BCA-689 (KF770899), BCA-698 (KF770900), CAI-133 (KF770895) and CAI-8 (KF770890), reported earlier to have biocontrol and PGP properties in chickpea (Alekhya and Gopalakrishnan, 2016) were further studied in this investigation.

### Greenhouse studies

All the seven *Streptomyces* spp. were evaluated for their PGP traits under greenhouse conditions. Soil mixture containing black soil,

sand and farm yard manure (3:2:1) was prepared and filled in plastic pots (8"). A total of eight treatments (seven *Streptomyces* spp. and a control; without any inoculum) each with three replications were maintained. Sorghum seeds (SPV1411; maturing in 125 to 128 days) were surface-sterilized with 2.5% chlorox for 5 min, rinsed 8-10 times with sterilized water and incubated with *Streptomyces* treatment ( $10^7$  cfu ml<sup>-1</sup>; grown in starch casein broth-SCB) for 1 h before sowing. In each pot, three seeds were sown and thinned to one after germination. At 15, 30 and 45 days after germination (DAS), a booster dose of *Streptomyces* spp. (5 ml per pot,  $10^7$  cfu ml<sup>-1</sup>) was applied on the soil together with watering. At 30 DAS, PGP parameters including the plant height, leaf area, leaf weight, shoot weight and root weight and length; and at 60 DAS, the plant height, leaf area, leaf weight, shoot weight and root weight were recorded. At final harvest, the panicle weight, seed weight, shoot weight and root weight were recorded.

### Field studies

Field trials were performed in 2012 Rabi (post-rainy) season at ICRISSAT, Patancheru (17°30.861'N; 78°16.080'E; altitude = 540 m) in the Telangana State of India. The experimental field soil is characterised as 51% clay, 22% silt and 26% sand with an organic carbon content of 0.4–0.5% and an alkaline pH of 7.5–8.1. Plots were composed of 4 × 3 m ridges arranged in a randomized complete block design (RCBD) with three replications. The seven selected strains of *Streptomyces* (BCA-546, BCA-659, BCA-667, BCA-689, BCA-698, CAI-133 and CAI-8) were grown in SCB for five days, soaked with sorghum seeds (SPV1411) just before sowing for 1 h and sown by hand at 5 cm depth. A booster dose of *Streptomyces* spp. ( $10^9$  cfu ml<sup>-1</sup>) was applied to soil at an interval of 15 DAS until flowering stage. The control plots contained no *Streptomyces* spp. Weeding was performed as and when required. No incidence of insect-pest or phytopathogens attack was observed during the cropping period. At 60 DAS, plant growth-parameters including the plant height, leaf area, root weight, shoot weight and leaf weight were recorded. During the final harvest, the growth and yield parameters including the plant height, panicle length, 1000 seed weight, grain yield and stover yield were recorded. Soil samples (from the 0 to 15 cm soil profile) were collected at flowering (60 DAS) and harvesting stages and analysed for organic carbon %, available P and total N using the standardized protocols described by Nelson and Sommers (1982), Olsen and Sommers (1982) and Novozamsky et al. (1983), respectively.

### Colonization studies

Sorghum root colonization by *Streptomyces* spp. was studied by scanning electron microscopy (SEM) as per the protocols of Gopalakrishnan et al. (2015a). In brief, the seeds of sorghum (SPV1411) were surface-sterilized with 2.5% chlorox for 5 min followed by 70% ethanol in water for 5 min and rinsed with sterilized water (several times). The sterilized seeds were allowed to germinate on a Petri dish containing blotter paper for two days under dark conditions. The germinated seeds were treated with *Streptomyces* spp. (BCA-546, BCA-659, BCA-667, BCA-689, BCA-698, CAI-8 and CAI-133;  $10^7$  cfu ml<sup>-1</sup>) for 1 h and sown in the pots containing sterilized coarse sand and incubated in a greenhouse for 15 days. At the end of the incubation, the root tips of the plants were fixed in 2.5% glutaraldehyde, 0.1 M phosphate buffer (pH 7.2) for 24 h at 4°C and post fixed in 2% aqueous osmium tetroxide for 4 h. The processed samples were mounted and coated with a thin layer of gold using an automated sputter coater (Model - JEOL JFC-1600) for 3 min and further scanned under SEM (Model: JOEL-JSM 5600) at RUSKA Lab, Rajendranagar, Hyderabad, Telangana, India.

**Table 1.** Effect of the seven *Streptomyces* spp. on the morphological observations of sorghum under greenhouse conditions at 30 days after sowing.

Strains	Plant height (cm)	Leaf area (m <sup>2</sup> cm)	Root length (m plant <sup>-1</sup> )	Root weight (g plant <sup>-1</sup> )	Shoot weight (g plant <sup>-1</sup> )	Leaf weight (g plant <sup>-1</sup> )
BCA-546	85.0	589	54.2	0.57	1.08	2.35
BCA-659	82.7	462	43.2	0.46	1.60	1.81
BCA-667	82.0	479	46.8	0.50	1.53	2.00
BCA-689	84.3	569	52.5	0.70	1.58	2.12
BCA-698	77.7	473	45.0	0.48	1.75	1.73
CAI-8	90.0	569	45.0	0.46	1.52	1.76
CAI-133	78.7	481	49.2	0.46	1.65	1.70
Control	77.3	454	42.3	0.45	1.49	1.68
LSD (5%)	6.12	76.5	7.21	0.057	0.152	0.413
CV%	4	9	9	6	5	12

The presented data are the averages of three replications; LSD= least significant difference; CV= coefficient of variation.

### Gene expression studies

All the seven *Streptomyces* spp. were grown in SCB broth and incubated at 28 ±2°C for five days. At the end of the incubation, the cultures were centrifuged at 10000 g, cell pellet was collected (500 mg) and RNA extracted using conventional Trizol method (Chomczynski and Mackey, 1995). The purity of extracted RNA was checked on agarose gel electrophoresis while the quality and quantity of RNA was estimated by Nanodrop (Thermo Scientific, Wilmington, USA) and RNA integrity by 2100 Bioanalyzer (Agilent, Redwood City, CA, USA). The RNA was diluted to 200 ng and cDNA was constructed. The quality and quantity of the cDNA was checked using Nanodrop and the concentrations were adjusted accordingly. Quantitative real-time polymerized chain reaction (qRT-PCR) was performed as per the manufacturer's instructions using Applied Biosystems 7500 Real Time PCR System with the SYBR green chemistry (Applied Biosystems, Foster City, CA, USA). IAA, siderophore and β-1,3-glucanase gene-specific primers for qRT-PCR were designed using primer 3 software (Rosen and Skaletsky, 2000). Genes relating to IAA (F: GTCACCGGGATCTTCTTCAAC; R: GATGTCGGGTGTTCTTGTCCAG), siderophore (F: ATCCTCAACACCCTGGTCTG; R: TCCTTGACTGGTACGGGACTT) and β-1,3-glucanase (F: CCGAACACCACCTACTCCAC; R: CCAGGTTGAGGATCAGGAAG) production were collected from UniprotKB database (<http://www.uniprot.org/uniprot>) as described in Gopalakrishnan et al. (2015a). PCR reactions and data analysis were done as described by Gopalakrishnan et al. (2015a).

### Statistical analysis

Data were analysed by using Analysis of Variance (ANOVA) technique (Genstat 10.1 version) to evaluate the different treatments and mean separations were done with LSD at significant levels of 1 and 5%.

## RESULTS

### Greenhouse studies

When the seven *Streptomyces* strains were evaluated for their PGP traits under greenhouse conditions,

considerable enhancement in the growth and yield parameters were observed. At 30 DAS, all the strains resulted in enhanced plant height (up to 14%), leaf area (up to 23%), root length (up to 22%), root weight (up to 36%), shoot weight (up to 17%) and leaf weight (up to 28%) than the un-inoculated control (Table 1). Similarly, treatments with *Streptomyces* led to growth enhancements than the un-inoculated control at 60 DAS, although the rate of increase was relatively lower. Among the seven tested strains of *Streptomyces*, BCA-546 and BCA-689 significantly enhanced most of the PGP traits including plant height, leaf area, leaf weight, root length, root weight, shoot weight, panicle weight and seed weight (Table 2).

### Field studies

When the PGP potentials of the seven *Streptomyces* strains were evaluated under field conditions, considerable enhancement in growth and yield parameters were observed in sorghum. At 60 DAS, the *Streptomyces* strains showed increased leaf area (up to 18%), leaf weight (up to 17%), stem weight (up to 11%) and root weight (up to 29%) while at final harvest, panicle length (up to 19%), 1000 seed weight (up to 7%), grain yield (up to 17%) and stover yield (up to 20%) than the un-inoculated control (Table 3). The soil mineral parameters including soil organic C (up to 12%), available P (up to 6%) and total N (up to 12%) were also found to be enhanced at both flowering and final harvest stages than the un-inoculated control (Table 4). Among the tested strains, three strains (BCA-546, BCA-659 and BCA-689) were found to consistently and significantly enhance growth parameters, grain and stover yields.

### Colonization studies

All the seven strains of *Streptomyces* showed extensive

**Table 2.** Effect of the seven *Streptomyces* spp. on the morphological and yield observations of sorghum under greenhouse conditions at 60 days after sowing and final harvest

Strains	60 days after sowing					At final harvest			
	Plant height (cm)	Leaf area (m <sup>2</sup> cm)	Root weight (g plant <sup>-1</sup> )	Shoot weight (g plant <sup>-1</sup> )	Leaf weight (g plant <sup>-1</sup> )	Panicle weight (g plant <sup>-1</sup> )	Seed weight (g plant <sup>-1</sup> )	Shoot weight (g plant <sup>-1</sup> )	Root weight (g plant <sup>-1</sup> )
BCA-546	146.0	2856	5.00	33.20	25.59	50.25	45.88	48.66	13.59
BCA-659	150.3	2781	4.85	33.06	25.68	50.98	46.16	47.88	14.88
BCA-667	148.3	2851	4.78	29.66	25.10	53.58	46.55	47.88	12.90
BCA-689	138.3	2712	5.53	29.96	25.39	57.65	50.11	49.27	14.84
BCA-698	149.3	2726	4.84	29.46	25.12	50.04	46.86	48.89	14.92
CAI-8	143.0	2671	4.88	29.06	25.17	49.87	45.45	47.89	12.00
CAI-133	144.3	2693	4.80	29.08	25.00	50.04	46.21	47.75	12.00
Control	133.7	2652	4.76	28.81	24.31	49.70	44.80	47.69	11.76
LSD (5%)	8.75	124.1	0.230	1.547	0.551	1.954	2.59	0.784	2.121
CV%	4	3	3	3	1	2	3	1	9

The presented data are the averages of three replications; LSD = least significant difference; CV = coefficient of variation.

**Table 3.** Effect of the seven *Streptomyces* spp. on the morphological and yield observations of sorghum under field conditions at 60 days after sowing and final harvest.

Strains	60 days after sowing					At final harvest				
	Plant height (m)	Leaf area (m <sup>2</sup> cm)	Root weight (g plant <sup>-1</sup> )	Shoot weight (g plant <sup>-1</sup> )	Leaf (g weight plant <sup>-1</sup> )	Plant height (m)	Panicle length (cm)	1000 seed weight (g)	Grain yield (t ha <sup>-1</sup> )	Stover yield (t ha <sup>-1</sup> )
BCA-546	1.96	2759	7.79	26.46	14.17	2.17	16.1	39.6	3.81	11.96
BCA-659	1.92	3331	10.91	25.08	16.55	2.08	17.2	40.3	4.11	13.75
BCA-667	1.95	2930	7.88	23.98	14.67	2.13	16.4	40.8	3.43	11.95
BCA-689	1.93	2936	7.80	26.04	16.45	2.16	15.6	40.6	3.52	11.20
BCA-698	1.91	2972	7.79	25.87	16.21	2.16	16.1	39.6	4.00	13.85
CAI-8	1.86	2798	7.82	24.34	15.20	2.29	16.7	40.4	3.74	12.50
CAI-133	1.89	2839	7.78	23.72	14.45	2.13	14.4	40.7	3.50	11.18
Control	1.88	2718	7.78	23.49	13.81	2.07	13.9	38.1	3.41	11.13
LSD (5%)	0.036	192.3	1.073	1.715	1.556	0.047	1.16	0.55	0.300	0.355
CV%	1	4	8	4	6	1	4	1	5	2

The presented data are the averages of three replications; LSD = least significant difference; CV = coefficient of variation.

colonization on the roots of sorghum. However, the extent of colonization was found to be most

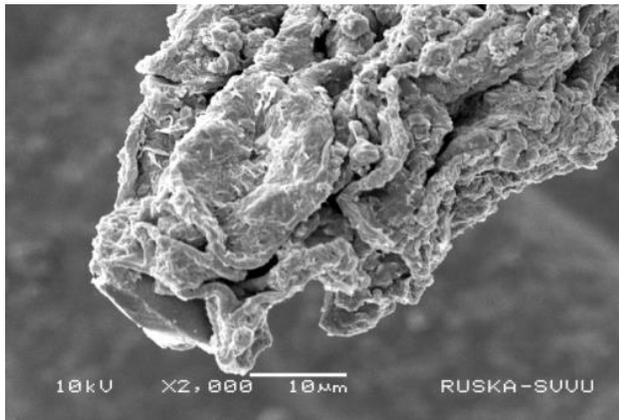
pronounced with BCA-546. Extensive mycelial growth penetrating the outer layer of the root was

noticed and also sporulation was observed in all the isolates, as compared to the un-inoculated

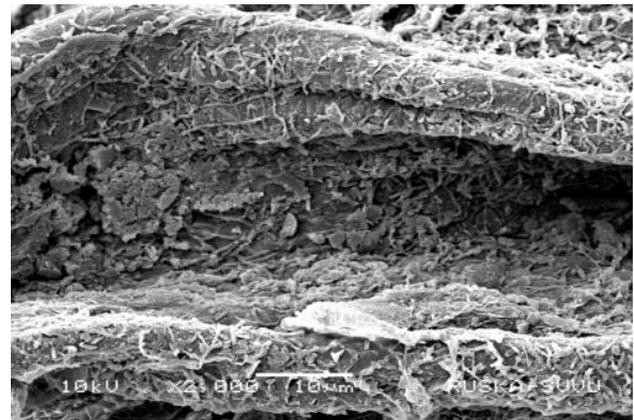
**Table 4.** Effect of seven *Streptomyces* spp. on the soil mineral properties of sorghum grown under field conditions at flowering and final harvest.

Strains	At flowering stage			At harvest stage		
	Total N (ppm)	Available P (ppm)	OC (%)	Total N (ppm)	Available P (ppm)	OC (%)
BCA-546	666	6.0	0.50	583	6.3	0.46
BCA-659	679	5.8	0.51	585	7.1	0.45
BCA-667	610	5.9	0.50	552	6.0	0.46
BCA-689	614	5.9	0.51	575	5.9	0.46
BCA-698	616	6.5	0.50	584	9.7	0.46
CAI-8	615	6.3	0.55	570	6.0	0.44
CAI-133	643	8.1	0.50	551	5.9	0.46
Control	605	5.8	0.49	535	5.9	0.44
LSD (5%)	39.9	0.37	0.022	21.6	0.41	0.009
CV%	3	3	2	2	3	1

The presented data are the averages of three replications; N = nitrogen; P = phosphorus; OC = organic carbon; LSD = least significant difference; CV = coefficient of variation.



Control



BCA-546

**Figure 1.** SEM photograph of BCA-546 strain showing extensive colonization on the roots of sorghum.

control (Figure 1).

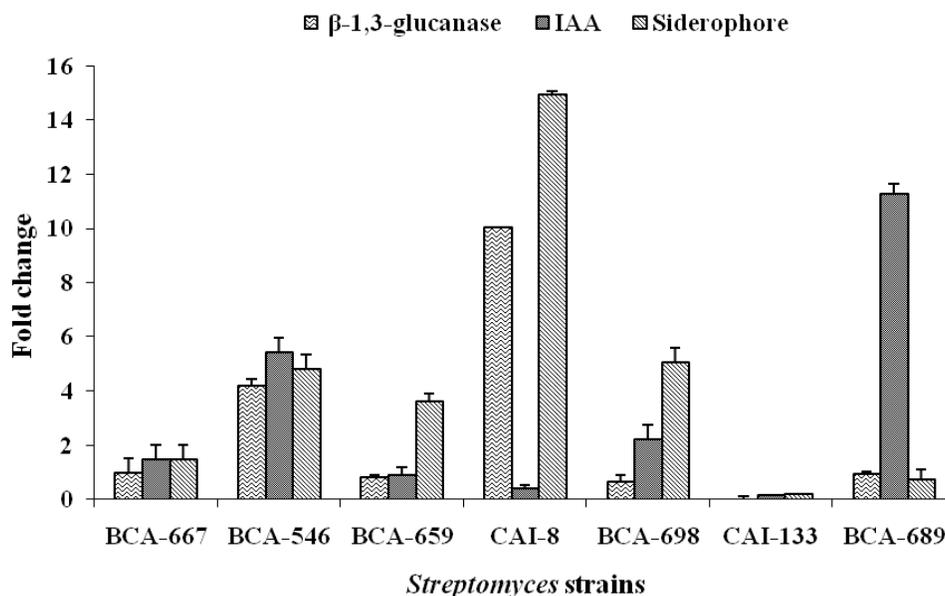
### Gene expression studies

The gene expression profiles of  $\beta$ -1,3-glucanase, IAA and siderophore genes for all the strains (except CAI-133) showed up-regulation. Among the seven strains,  $\beta$ -1,3-glucanase was up-regulated up to 10 fold, IAA by 11 fold and siderophore by 15 fold for CAI-8, BCA-689 and CAI-8, respectively (Figure 2).

### DISCUSSION

The major reasons for the lower yield in sorghum

includes fungal pathogens and unavailability of essential nutrients and iron to the plants (Davis and Bockus, 2001; Igual et al., 2001). PGP microbes including actinomycetes can play a vital role in enhancing the yields of sorghum. Most of the actinomycetes in soil belong to the genus *Streptomyces* and are reported to have potentials for PGP and biocontrol in many crops. It is reported that 60% of the biologically active compounds in agriculture such as antifungal, antibacterial and PGP substances are produced by *Streptomyces* spp. (Suzuki et al., 2000; Ilic et al., 2007; Khamna et al., 2010). In the present study, seven strains of *Streptomyces* having potential to produce PGP and biocontrol traits such as IAA, siderophores, lipase, cellulase, protease,  $\beta$ -1,3-glucanase, chitinase and hydrocyanic acid (Alekhya and Gopalakrishnan, 2016) were further studied for their PGP



**Figure 2.** Gene expression profiling of PGP genes of the seven *Streptomyces* strains.

traits in sorghum under greenhouse and field conditions. The results showed that all the *Streptomyces* strains enhanced the growth and yield parameters under both greenhouse and field conditions than the un-inoculated control plots when applied as seed coatings and soil inoculations. Among the seven *Streptomyces* strains studied, BCA-689, BCA-698, BCA-546, BCA-659 were found to be the best sorghum growth and yield promoting strains. They were also found to be the best strain which enhanced the soil mineral parameters including total N, available P and organic C as compared to the other *Streptomyces* strains. Hence, these *Streptomyces* strains may be promoted as inoculants for growth and yield enhancement in sorghum.

In the present study, under greenhouse, all the *Streptomyces* strains consistently enhanced root length and weights of sorghum (Table 1). The enhanced root length and mass will help the sorghum plants to absorb moisture and nutrients from the deeper zone of soil. This could be one of the reason why the yield and shoot and root biomass were found more in *Streptomyces* treated plots as compared to the control plots. The production of growth-promoting substances by PGP strains causes modifications in the morphology of roots, influencing nutrient and water absorption, and consequently promoting plant growth (Bashan and Holgum, 1997; Carla et al., 2008). Colonization observed in the present study adds further evidence to the effect of PGP microbes on root modifications. In the authors' previous study, these seven *Streptomyces* strains were also reported to be capable of producing several direct PGP traits including IAA and siderophore and indirect PGP traits including  $\beta$ -1,3-glucanase, chitinase and

hydrocyanic acid (Alekhya and Gopalakrishnan, 2016). Hence, these direct and indirect PGP traits of these strains could also be one of the reasons for the yield as well as shoot and root biomass enhancement of sorghum. PGP microorganisms enhance the plant growth directly by synthesis of phytohormones (Xie and Pasternak, 1996) or indirectly by preventing deleterious effect of pathogenic microorganisms, mostly due to the synthesis of antibiotics (Sivan and Chet, 1992). Actinomycetes are reported to promote plant growth by producing IAA which enhance the root growth or produce siderophores which enhance the nutrient uptake (Khamna et al., 2009). Actinomycetes including *Streptomyces* were previously reported for the control of plant fungal diseases and also enhance plant growth in cucumber, guava and tomato (El-Tarabily and Sivasithamparam, 2006; El-Tarabily et al., 2010; Shimizu, 2011; Mohandas et al., 2013; Sreeja and Surendra, 2013; Talwinder et al., 2013). PGP was also reported in sorghum using *Streptomyces* spp. (Alekhya and Gopalakrishnan, 2014; Gopalakrishnan et al., 2013a) and bacteria using *Pseudomonas fluorescens* and *Bacillus subtilis* under greenhouse conditions (Prathibha and Siddalingeshwara, 2013). In addition to their ability to inhibit plant pathogens, some actinomycetes are also known to form close associations with plants, colonize their internal tissues without causing disease symptoms, and promote their growth (Kunoh, 2002). The use of *Streptomyces* spp. for PGP in sorghum at field level has not been reported before, which makes the present study a novel approach for PGP in sorghum.

It is accepted that microorganisms effective as biocontrol and PGP agents must have good rhizosphere

competence, that is, have ability to colonize root of the host plant (Buell et al., 1991; Chiarini et al., 1998). In the present study, based on the SEM analysis, it was found that all the strains colonized the roots of sorghum. Beneficial actinomycetes were reported to colonize many host plants (Cao et al., 2005; Shi et al., 2009; Ruanpanun et al., 2010). *Streptomyces* spp. has been previously described as rhizosphere-colonizing bacteria (Miller et al., 1990a, b; Tokala et al., 2002). Hence, it is concluded that the selected strains of *Streptomyces* exhibited extensive colonization which correlates with their PGP properties.

In the present study, when the seven strains were evaluated for their gene expression profile, all strains (except CAI-133) up regulated  $\beta$ -1,3-glucanase, IAA and siderophore genes. The reason for selecting only  $\beta$ -1,3-glucanase, IAA and siderophore traits for expression profiles is that these three traits are directly linked to growth promotion of the plants. Similar results were also reported by Gopalakrishnan et al. (2013b, 2015a, b) which support the PGP by *Streptomyces* strains.

## Conclusion

In the present study, the seven selected *Streptomyces* spp. were found to enhance the growth of sorghum under both greenhouse as well as field conditions. These isolates were also found to have strong colonizing capability for the root surface of the sorghum plant and expressed PGP genes. Hence these isolates can be best employed for the PGP in sorghum. Further, the PGP and biocontrol potentials of the seven strains can be evaluated in other crops.

## Conflict of Interests

All the authors declare that they have no financial/commercial conflicts of interest.

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