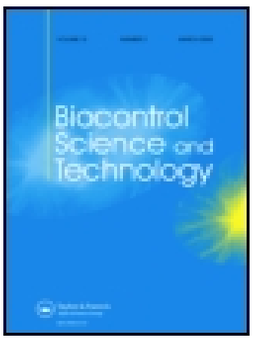

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Streptomyces sp. as plant growth-promoters and host-plant resistance inducers against *Botrytis cinerea* in chickpea

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ABSTRACT

Two hundred and fifty seven actinobacteria, isolated from five different rhizosphere soils of chickpea, were evaluated for their antagonistic potential against *Botrytis cinerea*, causal agent of *Botrytis* grey mold (BGM) disease in chickpea, by dual culture assay. Of them, three most promising isolates (ATIRS43, ATIRS65 and ARRS10) with highest inhibitory activity (67–77%) were identified as *Streptomyces* sp. These selected isolates induced growth of chickpea genotype JG11 as a consortium rather than an individual inoculum. Co-inoculation of the selected *Streptomyces* sp. with *Mesorhizobium ciceri* UPM-Ca7^T enhanced nodulation and nitrogenase activity in five chickpea genotypes (ICCV2, ICCV10, ICC4958, Annigeri and JG11). The selected *Streptomyces* sp. significantly reduced the disease incidence caused by *B. cinerea* by 28–47% over the un-inoculated control across the chickpea genotypes ICC4954 (susceptible), ICCV05530 (moderately resistant) and JG11 (unknown resistance). The *Streptomyces* sp. were also able to induce host-plant resistance, irrespective of the genotype, through the induction of various antioxidant enzymes and phenolics. Phenolic profiling of *B. cinerea*-affected and *Streptomyces* treated plants of ICCV05530 further confirmed host-plant resistance traits. This study indicated that the selected *Streptomyces* sp. have the potential for biological control of BGM disease in chickpea.

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Botrytis cinerea; *Streptomyces*; chickpea; plant growth-promotion; host-plant resistance; antioxidants

Introduction

Botrytis cinerea Pers. is a polyphagous fungal pathogen that infects over 200 crops covering dicots, monocots and ornamentals and causes *Botrytis* grey mold (BGM) disease (Dean et al., 2012). Being a necrotrophic fungus, it kills host plant cells by producing toxin (such as botrydial), lytic enzymes (such as chitinase and β-1,3-glucanase) and reactive oxygen species (ROS); such as peroxides and superoxide. It is considered as the second most key phytopathogen of great economic importance. In the context of chickpea, BGM is a crucial foliar disease that can cause complete yield loss in areas with high humidity and

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rains (Anuradha et al., 2011; Pande, Kishore, Upadhyaya, & Rao, 2006). Developing chickpea cultivars with high level of BGM resistance is a challenging task due to lack of BGM resistance counterparts in cultivated chickpea and a wider adaptability and high genetic diversity of *B. cinerea* posing a greater risk of host plant resistance breakdown (Pande et al., 2006). Consequently, there is no chickpea variety developed for BGM resistance till date. The only global protection strategy available for BGM is the use of fungicides costing about €1 billion/annum (Dean et al., 2012); however this leads to resistant strain development of the pathogen (Hahn, 2014).

Integrated disease management (IDM) is a greener alternative to the conventional use of pesticides where pesticides are used only when the disease incidence reach economic threshold levels. IDM promote the buildup of biocontrol agents (BCA) in the crop ecosystems. BCA are complex microbiomes of rhizosphere or plant's internal tissue that induce plant growth and protection against many stress factors through an array of mechanisms (Glick, 2012). Recent studies have documented that, these microbiomes also influence crop's functional profile (Flores-Félix et al., 2015). Extensive reports including field studies are available for microbiomes on a range of crops; however most of it belongs to the genus of *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter, Pseudomonas*, *Serratia*, *Paenibacillus*, *Rhizobium* and *Trichoderma*. However, reports for the phylum actinobacteria is limited, despite its ubiquitous existence in soil and plants and bioactive metabolites (Gopalakrishnan et al., 2011; Jog, Nareshkumar, & Rajkumar, 2012; Onaka, 2017). Several BCA had been reported to control BGM *in vitro* and *in planta* mainly on tomato, apple, grape and strawberries (Haidar, Fermaud, Calvo-Garrido, Roudet, & Deschamps, 2016); however, only a few studies have documented on chickpea viz, fungus *Gliocladium roseum* (Burgess, Bretag, & Keane, 1997), fungal metabolite citrinin (Sreevidya et al., 2015) and bacterium *Bacillus cereus* (Kishore & Pande, 2007). In view of aforesaid points, the present study was aimed to isolate antagonistic actinobacteria against *B. cinerea* and to further evaluate the mechanism behind plant growth-promotion and host-plant resistance particularly in chickpea.

Materials and methods

Sample collection and isolation of actinobacteria

Chickpea rhizospheric soil samples were collected during November 2014 across India (Supplementary Table 1). The samples were subjected to standard laboratory protocols (Gopalakrishnan et al., 2011) and plated on starch casein agar (SCA) and actinomycete isolation agar (AIA) supplemented with cycloheximide (50 μg ml^{-1}) and nystatin (25 μg ml^{-1}). The plates were incubated at 28°C for a week. The most prominent colonies were isolated and maintained on SCA/AIA slants at 4°C for further studies.

Primary screening by antibiosis

Actinobacterial isolates were screened for antibiosis property against *B. cinerea* (acquired from legumes pathology, ICRISAT Patancheru, India) by dual culture assay. The isolates were streaked on glucose casamino acid yeast extract (GCY) agar (on one edge of the plate; 1 cm from the corner) and incubated at 28°C for 2 days. At the end of incubation, a 6 mm fungal disc was placed on the other edge of the plate (1 cm from the corner) and incubated

at 19°C for a week. Inhibition of fungal mycelium (halo zone) around the actinomycete colony was noted as positive and the inhibition zone (diameter) measured and % inhibition calculated. The isolates with 50% and above inhibitory activity were characterised further.

In vitro biocontrol and plant growth-promoting traits

The selected actinobacteria were evaluated for the production of indole acetic acid (IAA; Patten & Glick, 1996), siderophore (Schwyn & Neilands, 1987), ß-1,3-glucanase (Singh, Shin, Park, & Chung, 1999), chitinase (Hirano & Nagao, 1988), cellulase (Hendricks, Doyle, & Hugley, 1995), lipase and protease (Bhattacharya, Chandra, & Barik, 2009), hydrocyanic acid (HCN; Lorck, 1948), ammonia (Cappuccino & Sherman, 1992) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Penrose & Glick, 2003). Solubilisation of phosphate (Fiske & Subbarow, 1925), potassium (Rajawat, Singh, Tyagi, & Saxena, 2016) and zinc (Saravanan, Kalaiarasan, Madhaiyan, & Thangaraju, 2007) were also determined.

Molecular identification of the actinobacteria

Molecular identification of the selected antagonistic actinobacteria against *B. cinerea* were done as per the protocols of Vijayabharathi et al. (2014) The nucleotide sequences of the selected antagonistic actinobacteria were submitted to GenBank and the NCBI GenBank accession numbers were obtained.

Pot experiments

The selected actinomycetes were evaluated *in planta* by three experiments under controlled environmental conditions at ICRISAT Patancheru, India. Experiment 1 was conducted during October 2015 to evaluate the plant growth-promoting (PGP) properties on chickpea genotype JG11; experiment 2 was conducted during November 2016 to evaluate the co-inoculation effects on nodulation with reference strain *Mesorhizobium ciceri* (ATCC 51585^{T}, ATCC, Manassas, VA, USA) on five chickpea genotypes ICCV2, ICCV10, ICC4958, Annigeri and JG11; and experiment 3 was conducted during May 2016 to evaluate the antagonistic potential against *B. cinerea* and induced host-plant resistance on chickpea genotypes ICC4954 (susceptible to BGM), ICCV05530 (moderately resistant to BGM) and JG11 (unknown BGM resistance). A randomised complete block design was used in all the experiments. The chickpea seeds were obtained from Gene Bank, ICRISAT Patancheru.

Experiment 1 – in planta plant growth responses

The experiment contained five treatments (control, three best antagonistic actinobacteria and their consortium) with six replications treatment^{-1}. Pot mixture was prepared with black soil, sand and farm yard manure (3:2:1), sterilised and filled in 8″ plastic pots. The chickpea seeds were surface sterilised (2.5% sodium hypochlorite for five min) and subjected to seed bacterization (10^{8} CFU ml^{-1} h^{-1}). The seeds were allowed to dry and sown in pots (four seeds/pot but thinned to two after a week). Booster doses of actinobacteria (5 ml seedling^{-1}, 10^{8} CFU ml^{-1}) were applied at 15 and 30 days after sowing (DAS)

by soil drench method. Observations including shoot length and dry weight, branch number, flower number, pod number, leaf area, SPAD and root length, surface area, volume and dry weight were determined at 45 DAS while at harvest, seed number, seed weight, pod number, pod weight and total biomass were recorded.

Experiment 2 – in planta co-inoculation effect

It was conducted with five treatments (control, *M. ciceri*, best antagonistic isolate 1 + *M. ciceri*, best antagonistic isolate 2 + *M. ciceri* and best antagonistic isolate 3 + *M. ciceri*) and six replications treatment^{-1}. The chickpea seeds of ICCV2, ICCV10, ICC4958, Annigeri and JG11 were used. Pot mixing, seed surface sterilisation, seed bacterization and sowing were done as in experiment 1 and according to the treatments. *M. ciceri* was grown in yeast extract mannitol broth at 200 rpm, 28°C for 5–7 days with the cell count of $\sim 1 \times 10^9$ CFU ml^{-1}. For co-inoculation treatments, a cocktail consisting of *M. ciceri* and selected actinobacteria in the ratio of 1:1 was used (Egamberdieva, Berg, Lindström, & Räsänen, 2010). Inoculation of actinobacteria alone didn't produce any nodules in experiment 1, so they were not included in this experiment. Booster dose of inoculum was added at 15 DAS by soil drench method. Plant growth responses were determined by shoot dry weight, root dry weight and nodule dry weight at 35 DAS. Nitrogenase activity was estimated by acetylene reduction (μmol C_2H_4 plant^{-1} h^{-1}) activity in Hewlett Packard gas chromatograph (HP4890D), with FID detector, HP-PLOT-Q column and N_2 as carrier gas.

Experiment 3 – in planta antagonistic potential against B. cinerea

Inoculum of *B. cinerea* (containing conidia of 3×10^5 ml^{-1}) was prepared using marigold (*Tagetes erecta* L.) flowers (Pande et al., 2006). The chickpea seeds were planted in rows in plastic trays (30 × 20 × 5 cm) filled with sterilised sand and vermiculate (4:1) including one row of susceptible cultivar JG62 as a check. Ten days after germination, the seedlings were transferred in to a growth room maintained at 15 ± 2°C with approximately 1,500 lux light intensity and 12 h photoperiod. The experiment was conducted with three replications and each replication consisted of 10 seedlings. Commercially available *Trichoderma harzianum* (ECOSOM®-TH) was used as standard reference strain. A total of seven treatments were applied (T1: control, T2: disease control (*B. cinerea* challenged), T3: best antagonistic isolate 1 treated, T4: best antagonistic isolate 2 treated, T5: best antagonistic isolate 3 treated, T6: consortium of best antagonistic isolates 1 + 2 + 3 treated and T7: *T. harzianum* treated). In treatments T3–T7, the test inoculum (selected actinobacteria) were sprayed first and allowed to dry; later sprayed with the *B. cinerea* inoculum. Growth room was maintained with 24 h of 100% RH for first 10 days after pathogen inoculation (DAPI) followed by 8 h of 100% RH for another 2 days. BGM disease symptoms were recorded at 2 days interval until 12 DAPI. Disease severity was recorded following 1–9 rating scale (Anuradha et al., 2011) and the area under disease progress curve (AUDPC) was calculated (Shaner & Finney, 1977).

For evaluating host plant resistance traits, chickpea leaves were collected in liquid nitrogen from all the three replications at 4, 6, 8, 10 and 12 DAPI and stored at −80°C for further analysis. The oxidative damage to lipids was determined as malondialdehyde (MDA; Zhou & Leul, 1998) content and the results were expressed as nMoles MDA g^{-1}

fresh weight. The antioxidant enzymes studied include superoxide dismutase (SOD; Martinez, Marcelo, Oliva, & Moacyr, 2001), catalase (CAT; Aebi, 1984), ascorbate peroxidase (APX; Nakano & Asada, 1981), guaiacol peroxidase (GPX; Srivastava & Van Huystee, 1977), glutathione reductase (GR; Schaedle & Bassham, 1977), phenyl alanine ammonia lyase (PAL; Brueske, 1980) and polyphenol oxidase (PPO; Gauillard, Richardforget, & Nicolas, 1993). Total phenolic content (TPC) of the leaf samples were estimated by Folin-Ciocalteu method as per the protocols of Singh, Sarma, Upadhyay, and Singh (2013). The results were expressed as mg gallic acid equivalents (GAE) g^{-1} FW.

For phenolic profiling, fresh tissue of 500 mg of chickpea leaflets, harvested at 6 DAPI, was extracted with 10 ml of 50% methanol. The solvent was evaporated under reduced pressure (Buchi Rota Vapor, R215, Buchi, Switzerland) and the residue was dissolved in methanol (HPLC grade). HPLC (Waters made with quaternary pump and photodiode array detector (Model 2996) using Sunfire C_{18} column; RP, 5 µm pore size, 250 × 4.6 mm) was performed under gradient flow conditions with the mobile system consisting of 90% solvent A (2% acetic acid in acetonitrile) and 10% solvent B (2% acetic acid in HPLC grade water) and changing to 50% solvent A in 20 min and finally to 90% in 40 min (Singh, Jain, Sarma, Upadhyay, & Singh, 2014). The solvent flow rate used was 1 ml min^{-1}. Data acquisition and processing were performed via Empower 3 software 7.10.00.00. Phenolics (µg g^{-1} fresh weight) were estimated from the obtained peak area by comparing with the standards.

For lignin staining, chickpea plants were harvested at 6 DAPI. Free hand stem cross sections were cut and treated with phloroglucinol-HCL stain and observed under light microscope. The images were digitalised by Axiocam 105 colour, Scope.1, (Axioscope, Zeiss). Presence of lignin was indicated by red-violet colour (Jensen, 1962).

Statistical analysis

The data of (i) *in-vitro* PGP traits, (ii) chickpea growth responses, and (iii) disease incidence and antioxidant parameters of *in planta* anti-*B. cinerea* studies were subjected to one-way analysis of variance (ANOVA) and the significant difference between mean values was determined by Tukey's and Dunnett's test using Statistical Package for the Social Sciences (SPSS) 13.0 (SPSS Inc., Chicago, Illinois, USA). Pearson correlation coefficient has been calculated for *in vitro* and *in planta* growth promoting traits, and *in planta* antioxidant parameters using SPSS. Principal component analysis (PCA) on chickpea growth responses and antioxidant parameters has been done using R statistical package 3.2.5 (R Foundation for Statistical Computing). Significant relationship between the antioxidant parameters in the context of genotype, treatment, time and its interactions were tested through one-way and two-way ANOVA by GENSTAT 14.0 (VSN International Ltd., Hemel Hempstead, UK).

Results

Antibiosis and in vitro biocontrol traits

Chickpea rhizospheric soil samples collected from five states of India covering southern and northern states (Supplementary Table 1) yielded 257 actinobacteria in which 10 isolates showed above 50% inhibitory effect on *B. cinerea* in the dual culture assay. The ability of the ten isolates to produce extracellular lytic enzymes, volatiles and siderophores are

presented in Table 1. Among them, isolates ATIRS43, ATIRS65 and ARRS10 showed highest inhibitory activity of 77%, 70% and 67%, respectively and were able to produce HCN, ammonia (except ATIRS65), ß-1,3-glucanase, chitinase, cellulase (except ATIRS 65), protease, lipase and siderophore (except ATIRS65) (Table 1 and Supplementary Figure 1). The selected isolates were found to be compatible and showed 85% inhibitory activity when used as consortium (data not shown).

In vitro PGP traits

All the 10 actinomycetes produced IAA in the presence of L-tryptophan with the highest activity by ATIRS43 (62 µg ml^{-1}) followed by ATIRS23 (33 µg ml^{-1}) whereas the remaining isolates produced IAA between 1.3 and 5.8 µg ml^{-1}. The 10 isolates were also found to solubilise phosphate of 33–54 P equivalents µg ml^{-1}. Qualitative analysis of K solubilisation showed 30–143% efficiency in five isolates, i.e. ATIRS43, ATIRS65, ATIRS31, ATIRS23 and ARRS29. The selected isolates showed different pattern of Zn solubilisation for ZnP, ZnO and ZnC with an efficiency of 130–240%. In the current study, only two strains ATIRS43 and ATIRS18 were identified to produce ACC deaminase (Table 2).

Molecular identification of the promising antagonistic actinobacteria

Phylogenetic analysis of 16S rDNA sequences of the three most promising antagonistic actinobacteria, ATIRS43, ATIRS65 and ARRS10, matched with genus *Streptomyces* (Supplementary Figure 2). The sequences were submitted to GenBank, NCBI and accession numbers obtained as *Streptomyces* sp. ATIRS43 (MF276633), *Streptomyces* sp., ATIRS65 (MF276632) and *Streptomyces* sp., ARRS10 (MF276631).

In planta PGP activity of selected actinobacteria on chickpea

At 45 DAS, consortium, ARRS10 and ATIRS43 treated plants significantly enhanced shoot traits including shoot length, dry weight and branch number and root traits including root length and surface area over the un-inoculated control plants; whereas root volume and dry weight were found to be enhanced only in consortium treatment. Plants treated with ATIRS65 enhanced none of the shoot and root traits. Among the reproductive traits, only flower number was found to be significantly enhanced by consortium treatment. At harvest, consortium treatment showed significant enhancement on seed number (13.3 *vs.* 10 Plant^{-1}), seed weight (8.4 *vs.* 6.8 g Plant^{-1}), and total biomass (10.8 *vs.* 8 g Plant^{-1}) over the un-inoculated control plants. Though individual inoculations of ATIRS43 and ARRS10 showed some significant growth responses at 45 DAS, these growth responses turned out to be insignificant at harvest (Tables 3 and 4).

PCA and correlation analysis for in planta PGP responses

PCA analysis was done to get an overview of the effect of actinobacterial treatment on chickpea growth responses. The traits including shoot dry weight, SPAD, root surface area, flower number and seed number were used for this analysis. PCA for the selected chickpea growth responses showed the contribution of 67% and 23% variance by PC1 and PC2, respectively

Table 1. In vitro bio-control traits of the ten chickpea rhizospheric actinobacteria against B. cinerea.

Isolates	Antibiosis	Volatiles		Competitive molecules	Extracellular lytic enzymes				
		HCN	Ammonia¶	Siderophore "	β-1,3-glucanase*	Chitinase	Cellulase	Protease	Lipase
ATIRS43	77 ± 3^{a}	2	+	26.8 ± 1.7^{b}	14.8 ± 0.3^{b}	4	2	2	5
ATIRS65	70 ± 2^{ab}	2	–	–	13.6 ± 0.2^{c}	3	–	5	4
ARRS10	67 ± 2^{b}	1	+	51.7 ± 2.8^{a}	12.4 ± 0.6^{d}	3	4	3	3
ATIRS18	58 ± 2^{c}	1	+	24.1 ± 1.4^{b}	5.4 ± 0.3^{f}	4	–	2	3
ATIRS31	57 ± 3^{c}	1	+	–	16.6 ± 0.3^{a}	2	–	4	–
ARRS26	54 ± 10^{c}	1	–	–	5.0 ± 0.1^{g}	1	3	2	3
ARRS30	53 ± 7^{c}	1	–	–	9.2 ± 0.1^{e}	4	2	1	4
ATIRS23	51 ± 9^{c}	1	+	10.8 ± 0.6^{c}	4.7 ± 0.2^{g}	1	2	3	5
ARRS29	51 ± 3^{c}	1	–	–	4.1 ± 0.1^{h}	2	–	1	–
ARRS33	50 ± 2^{c}	1	–	–	12.3 ± 0.2^{d}	–	2	1	1

Values are Mean ± SE ($n = 3$). Different superscript lowercase letters in same column indicates significant difference ($p < 0.05$) as per Tukey's test.

¶ – % inhibition; HCN-Hydrocyanic acid; " – % Units; * – Units, One unit of β-1,3-glucanase activity is defined as the amount of enzyme that liberated 1 µmol of glucose h^{-1} at defined conditions; + and – indicates positive and negative for ammonia production.

For HCN production, the following ratings scale was used: 0 = no colour change; 1 = light reddish brown; 2 = medium reddish brown and 3 = dark reddish brown. The rating scale for chitinase, cellulase, protease and lipase were as follows: 0 = no change; 1 = halo zone of 1–6 mm; 2 = halo zone of 7–12 mm; 3 = halo zone of 19–24 mm and 4 = halo zone of 25–30 mm and above.

Table 2. In vitro PGP traits of the ten antagonistic actinobacteria.

	Growth hormones	Mineral mobilisation					Stress reliever
				Zn*			
Isolates	IAA"	P¶	K*	ZnP	ZnO	ZnC	ACC deaminase#
ATIRS43	62.3 ± 1.2^{a}	43.9 ± 2.8^{cd}	30 ± 6^{c}	–	240 ± 6^{a}	–	3.6 ± 0.3
ATIRS65	3.9 ± 0.3^{d}	43.5 ± 0.3^{cd}	75 ± 3^{b}	130 ± 10^{c}	–	–	–
ARRS10	3.0 ± 0.3^{ef}	41.7 ± 0.1^{de}	–	–	–	170 ± 12^{b}	–
ATIRS18	1.3 ± 0.2^{g}	42.6 ± 0.5^{cd}	–	–	–	167 ± 3^{b}	2.9 ± 0.1
ATIRS31	3.3 ± 0.2^{def}	43.6 ± 1.0^{cd}	143 ± 15^{a}	187 ± 41^{ab}	217 ± 26^{ab}	233 ± 7^{a}	–
ARRS26	3.7 ± 0.3^{de}	41.3 ± 0.3^{e}	–	137 ± 7^{c}	163 ± 13^{c}	–	–
ARRS30	2.6 ± 0.2^{f}	45.0 ± 0.9^{c}	–	–	–	–	–
ATIRS23	33.3 ± 0.5^{b}	49.6 ± 1.8^{b}	113 ± 9^{a}	–	–	–	–
ARRS29	1.4 ± 0.1^{g}	33.2 ± 0.8^{f}	46 ± 5^{c}	217 ± 24^{a}	–	213 ± 12^{a}	–
ARRS33	5.8 ± 0.3^{c}	54.5 ± 0.5^{a}	–	–	183 ± 3^{bc}	–	–

Values are Mean ± SE ($n = 3$). Different superscript lowercase letters in same column indicates significant difference ($p < 0.05$) as per Tukey's test.

IAA – indole acetic acid; ZnP – zinc phosphate; ZnO – zinc oxide; ZnC – zinc carbonate; ACC – 1-amino cyclo propane-1-carboxylic acid; " – μg ml^{-1}; ¶ – P equivalents μg ml^{-1}; * – % Solubilisation efficiency; **#** – nmoles α-ketobutyrate mg $protein^{-1}$ h^{-1}.

(Supplementary Figure 3). PC1 was associated with effect of microbial treatment as the highest difference was observed between un-inoculated control and consortium; whereas PC2 depicts the effect of individual treatments as the highest difference was observed between ATRIS65 and ATIRS43 treatment. This observation further confirmed the results depicted in Tables 3 and 4. Correlation analysis indicated positive relationships of seed number with IAA, siderophore, P and Zn solubilisation, ACC d and HCN (Supplementary Table 2). Other traits such as SPAD, shoot dry weight, root surface area, flower number were also positively correlated with *in vitro* PGP traits. Besides this, correlation data between the groups provides concrete support to the microbial action towards chickpea growth responses (Supplementary Tables 3–7).

Co-inoculation effect of selected actinobacteria on nodulation in chickpea

Co-inoculation of actinobacteria, ATIRS43, ATIRS65 and ARRS10, with *M. ciceri* induced nodulation and nitrogenase activity irrespective of the chickpea genotypes, over the un-inoculated control plants with 0.03–0.08 mg $Plant^{-1}$ and 0.35–1.85 μmol C_2H_4 $Plant^{-1}$ h^{-1}, respectively (Figure 1). In un-inoculated control plants of ICCV2, Annigeri and JG11, there were some minute nodule like structures observed; however, they were devoid of nitrogenase activity. Among the co-inoculation treatments, significant increase in nodulation and nitrogenase activity over *M. ciceri* treatment was seen only in ICCV10 and JG11. This was further confirmed by ANOVA indicating the influence of *M. ciceri* and actinobacterial co-inoculation effects (Supplementary Table 8). Two-way ANOVA between genotype and treatment indicates the suitability of all microbial treatments for enhanced nodulation irrespective of the genotypes.

Antagonistic potential of selected actinobacteria against B. cinerea and induced host-plant resistance in chickpea

Effect on disease severity

AUDPC was found highest in ICC4954 (75) followed by JG11 (65) and ICCV05530 (55). This indicates that JG11 has medium resistance level to BGM (Figure 2). The microbial treatments significantly reduced the disease severity by 28–47% over the disease control

Table 3. PGP effect of selected actinobacteria on chickpea genotype JG11 under glasshouse conditions- at 45 DAS.

	Shoot traits			Leaf traits		Root traits				Reproductive traits	
Treatments	Length (cm)	Dry weight (g)	Branch number	Area (cm^2)	SPAD	Length (cm)	Surface area (cm^2)	Volume (cm^3)	Dry weight (g)	Flower number	Pod number
ATIRS43	29.1* ± 1.3	1.5 ± 0.2	9.4* ± 2.3	175.0 ± 27.5	59.8 ± 4.0	2546.9* ± 300.7	557.5* ± 55.2	9.8 ± 1.0	0.8 ± 0.2	9.7 ± 2.4	1.4 ± 1.3
ATIRS65	28.4 ± 1.8	1.5 ± 0.1	9.8* ± 1.6	191.0 ± 19.7	65.9 ± 2.8	2274.9 ± 198.7	512.2 ± 34.6	9.2 ± 0.5	0.8 ± 0.1	6.8 ± 2.1	1.8 ± 0.5
ARRS10	29.5* ± 0.9	1.6* ± 0.1	10.1* ± 0.9	187.5 ± 12.1	65.8 ± 2.0	2491.3* ± 143.4	558.2* ± 23.6	10.0 ± 0.4	0.7 ± 0.1	9.5 ± 2.3	1.1 ± 0.4
Consortium	30.2* ± 0.7	1.5* ± 0.1	10.8* ± 0.4	188.6 ± 24.4	69.0* ± 4.2	2733.8* ± 173.8	597.9* ± 26.0	10.5* ± 0.5	0.9* ± 0.0	11.0* ± 0.9	1.2 ± 0.6
Control	26.4 ± 0.9	1.2 ± 0.1	6.0 ± 0.5	154.3 ± 14.2	59.7 ± 3.5	1898.3 ± 167.3	461.7 ± 36.9	8.7 ± 0.7	0.7 ± 0.1	6.7 ± 0.9	0.8 ± 0.3

Values are Mean ± SE ($n = 6$). *Values are statistically significant over the control as per Dunnett's test ($p < 0.05$).

ATIRS43 – *Streptomyces* sp., ATIRS43 treated; ATIRS65 – *Streptomyces* sp., ATIRS-65 treated; ARRS10 – *Streptomyces* sp., ARRS-10 treated; Consortium – Consortium of ATIRS43 + ATIRS65 + ARRS10 treated.

Table 4. PGP effect of selected actinobacteria on chickpea genotype JG11 under glasshouse conditions-at harvest.

Treatments	Seed number (Plant^{-1})	Seed weight (g Plant^{-1})	Pod number (Plant^{-1})	Pod weight (g Plant^{-1})	Total biomass (g Plant^{-1})
ATIRS43	12.3* ± 1.1	7.9 ± 0.4	15.0* ± 1.2	7.6* ± 0.36	7.1* ± 0.4
ATIRS65	8.7 ± 0.6	6.1 ± 0.7	10.5 ± 0.6	5.4 ± 0.3	6.4 ± 0.3
ARRS10	9.5 ± 0.6	6.5 ± 0.6	11.5 ± 0.6	6.0 ± 0.3	6.5 ± 0.3
Consortium	13.3* ± 0.7	8.4* ± 0.6	14.8* ± 0.8	7.6* ± 0.4	10.8* ± 0.4
Control	10.0 ± 0.8	6.8 ± 0.4	11.7 ± 0.8	6.2 ± 0.3	8.0 ± 0.1

Values are Mean ± SE ($n = 6$). *Values are statistically significant over the control as per Dunnett's test ($p < 0.05$).
ATIRS43 – *Streptomyces* sp., ATIRS43 treated; ATIRS65 – *Streptomyces* sp., ATIRS-65 treated; ARRS10 – *Streptomyces* sp., ARRS-10 treated; Consortium – Consortium of ATIRS43 + ATIRS65 + ARRS10 treated.

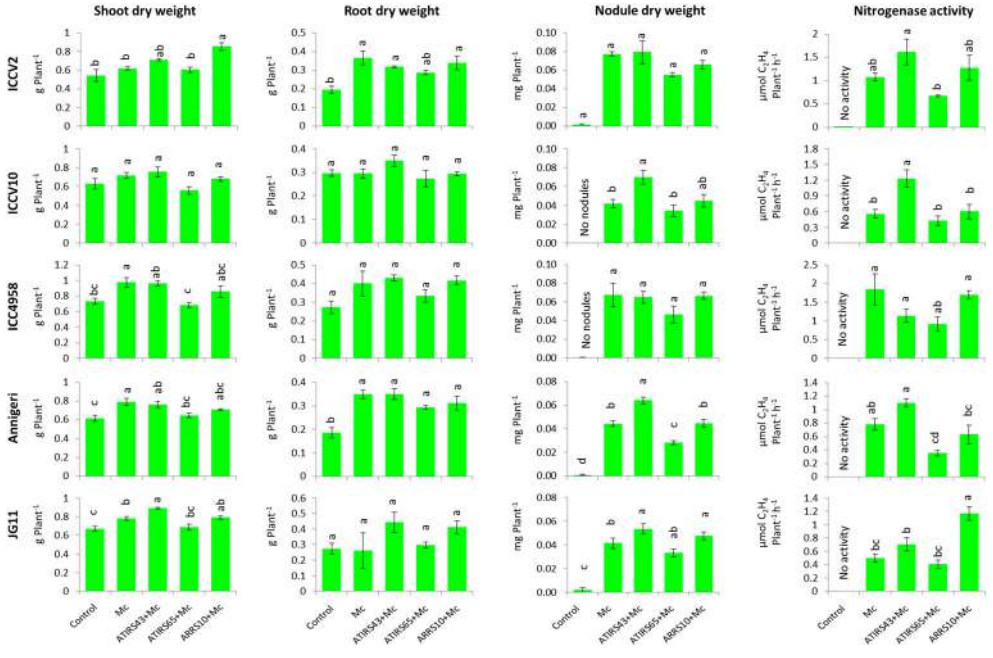

Figure 1. Effect of selected actinobacterial co-inoculation with *M. ciceri* on nodulation of different genotypes of chickpea. Values are Mean ± SE ($n = 6$). Error bar indicates SE. *Mc* – *M. ciceri* ATCC 51585^{T} treated. ATIRS43 – *Streptomyces* sp., ATIRS43 treated. ATIRS65 – *Streptomyces* sp., ATIRS-65 treated. ARRS10 – *Streptomyces* sp., ARRS-10 treated. Bars within a graph not sharing the same letter are significantly different as per Tukey's test ($p < 0.05$).

treatment, in which consortium and *T. harzianum* showed equal effectiveness irrespective of the genotypes; followed by single inoculations (Figure 2).

Effect on primary antioxidants

MDA was found to increase over time and highest at 12 DAPI in *B. cinerea* challenged plants irrespective of the genotypes studied followed by selected microbe treated and un-inoculated control plants. The consortium and *T. harzianum* treatments registered the least MDA content (2.1 fold less) over the control plants (Supplementary Figure 4).

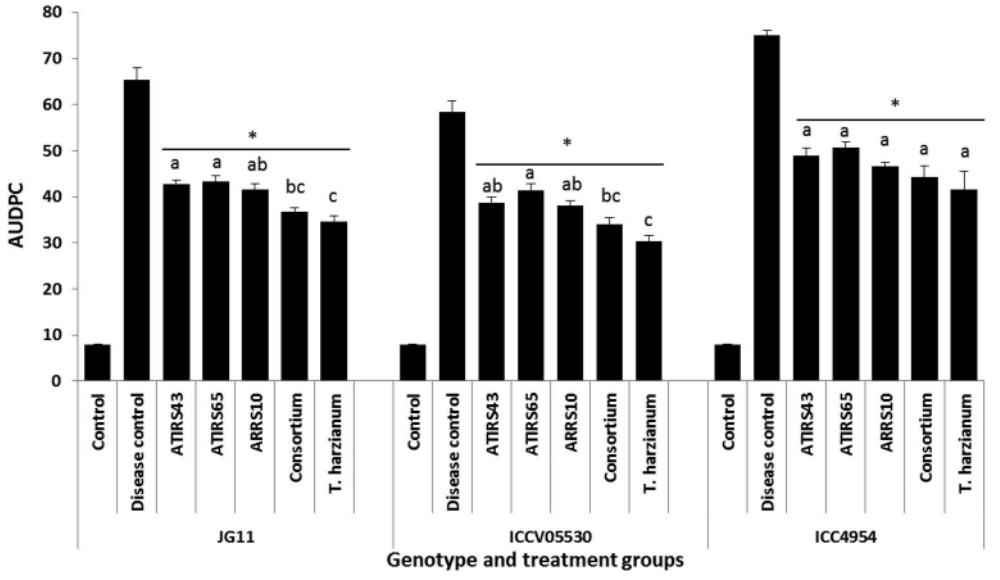

Figure 2. Effect of selected actinobacteria and *T. harzianum* on *B. cinerea* of different genotypes of chickpea. Values are Mean ± SE ($n = 3$). Error bar indicates SE. *Values are statistically significant against disease control as per Dunnett's test ($p < 0.05$). Bars having different lowercase letters indicate the significant difference among the microbial treatment groups as per Tukey's test ($p < 0.05$). ATIRS43 – *Streptomyces* sp., ATIRS43 treated; ATIRS65 – *Streptomyces* sp., ATIRS-65 treated; ARRS10 – *Streptomyces* sp., ARRS-10 treated; Consortium – Consortium of ATIRS43 + ATIRS65 + ARRS10 treated; *Th* – *Trichoderma harzianum* treated.

Un-inoculated control plants recorded minimum level of SOD activity (3–12 U g^{-1} FW) followed by disease control (5–19 U g^{-1} FW) and selected microbe treated plants (8–39 U g FW^{-1}) during the 12 day period (Supplementary Figure 5). A gradual increase in SOD was observed on selected microbial treatments reaching the highest on 10 DAPI in JG11 and ICC4954; and 12 DAPI in ICCV05530. CAT activity was found higher in ICCV05530 (96–415 U g^{-1} FW) followed by JG11 (89–405 U g^{-1} FW) and ICC4954 (64–337 U g^{-1} FW) genotypes. Consortium and *T. harzianum* treatments recorded gradual increase of CAT until 12 DAPI than single inoculations (Supplementary Figure 6).

APX activity was found to be induced by microbial treatments in response to *B. cinerea* up to 2.7, 3.4 and 3.7 fold in ICCV05530, JG11 and ICC4954 genotypes, respectively (Supplementary Figure 7). Peak GPX activity in disease control plants was noticed on 6 DAPI in JG11 and 8 DAPI in ICCV05530 and ICC4954; and at later periods it declined lesser than un-inoculated control plants. Microbial treatments induced GPX activity up to 1.7–2.6 folds than control plants, in which consortium and *T. harzianum* treated plants recorded significantly higher activity irrespective of the sampling time and genotype (Supplementary Figure 8). GR activity was found higher in ICCV05530 and JG11 (18–52 and 13–50 U g^{-1} FW, respectively) whereas it was significantly lower in ICC4954 (6–13 U g^{-1} FW) during the 12 day study period (Supplementary Figure 9).

PAL activity was found higher in all microbial treatments (an increase of up to 1.4 folds until 12 DAPI) over the un-inoculated control plants, whereas disease control group

recorded significantly decreased activity. All the three genotypes showed nearly similar PAL activity levels of 5–6.1, 5.2–5.6 and 4.5–5.2 mMol *t*-CA g^{-1} FW in JG11, ICCV05530 and ICC4954 genotypes, respectively (Supplementary Figure 10). PPO activity was found higher in ICCV05530 and JG11 (0.5–2.5 U g^{-1} FW, 0.3–1.9 U g^{-1} FW) genotypes while it was lower in ICC4954 (0.3–0.7 U g^{-1} FW) genotype. However, no significance was observed in respect to treatment and time (Supplementary Figure 11).

Effect on TPC and phenolic profiling

Both the actinobacteria and *T. harzianum* treatments have significantly increased the TPC (11–69 mg GAE g^{-1} FW) over un-inoculated control plants (5–8 mg GAE g^{-1} FW). Irrespective of the genotypes; TPC and sampling time were found to be directly proportional in consortium and *T. harzianum* treatments. *B. cinerea* challenged treatment also increased TPC but the values were intermittent between control and microbial treated plants (Supplementary Figure 12).

In order to observe the difference in individual phenolics, in the selected microbial, control, disease control and consortium treatments, ICCV05530 genotype was analyzed for phenolic profiling, which showed qualitative and quantitative changes in different treatments (Figure 3). For instance, control plants alone shown to have 3,4-dihydroxy flavonone; phloretic acid increased up to 3.5 and 6.8 fold in *B. cinerea* challenged and consortium treatments, respectively over the control plants. Among the phenolics tested, phloretic acid, quercetin, formononetin and kaempferol were observed in both *B. cinerea* challenged and consortium treated plants but with varying concentrations as 66866 *vs.* 128332 µg g^{-1} FW, 30859 *vs.* 5814 µg g^{-1} FW, 10912 *vs.* 1924 µg g^{-1} FW and 5189 *vs.* 612 µg g^{-1} FW, respectively. Additional peaks of chlorogenic acid, sinapic acid and ferulic acid were observed in consortium treated plants (Supplementary Table 9).

PCA and correlation analysis for in planta antioxidant responses

To understand the effect of selected microbial treatments on antioxidant activities with respect to genotype, the tested parameters were subjected to PCA (Figure 4 and Supplementary Figure 13). PCA on Figure 4 for all the three chickpea genotypes indicate the overall variance of 80% for PC1 (57%) and PC2 (23%). PC1 was associated with effect of pathogen challenge as the highest difference observed between un-inoculated control and disease control group; whereas PC2 was associated with the effect of microbial treatment, as the highest difference being observed on *T. harzianum* treatment and *B. cinerea* challenge. Resistance level observed in AUDPC and the tested antioxidant parameters for JG11 is being confirmed here as JG11 stands between ICCV05530 (moderately resistant to BGM) and ICC4954 (susceptible to BGM) genotypes. Effect of *B. cinerea* in collapsing the antioxidant system was indicated through disease control groups, as they fall ahead of threshold level irrespective of the genotypes. The relatedness between the antioxidant enzymes is clearly documented by the arrangement of arrows in Figure 4 and by the correlations in Table 5. PCA on effect of microbial treatments with respect to each genotype is depicted in Supplementary Figure 13(i, ii & iii) respectively for

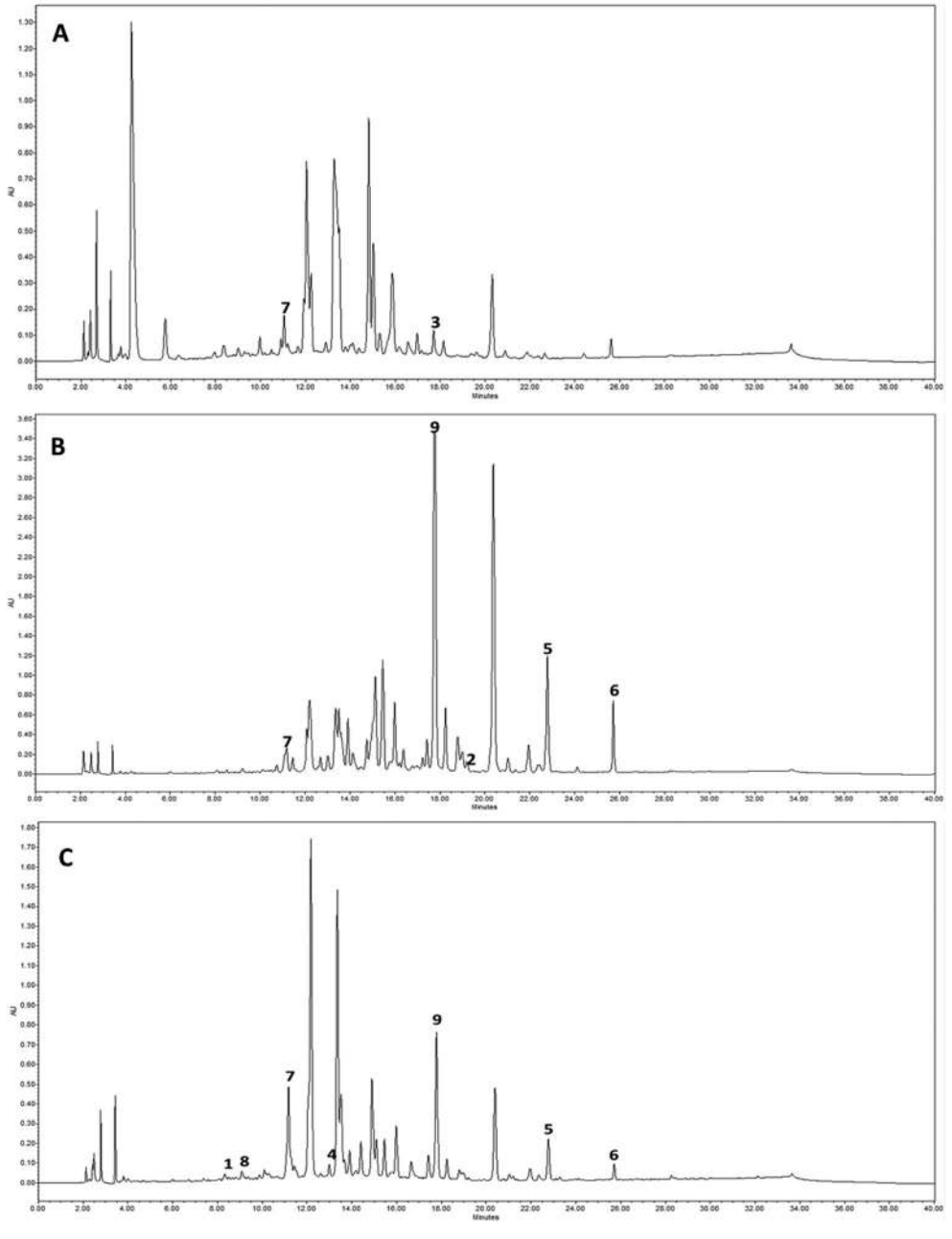

Figure 3. Phenolic profiling of MR chickpea genotype ICCV05530 towards *B. cinerea* challenge and consortium treatment. A – Control; B – disease control (*B. cinerea* challenged); C – Consortium of ATIRS43 + ATIRS65 + ARRS10 treated; 1 – Chlorogenic acid; 2 – Cinnamic acid; 3–3, 4-dihydroxy flavonone; 4 – Ferulic acid; 5 – Formononetin; 6 – Kaempferol; 7 – Phloretic acid; 8 – Sinapic acid; 9 – Quercetin.

JG11, ICCV05530 and ICC4954 genotypes. All these PCA gives an overview that, antioxidant enzymes increases with increase in time to some extent; and consortium and *T. harzianum* exhibits higher antioxidant activity than single inoculations.

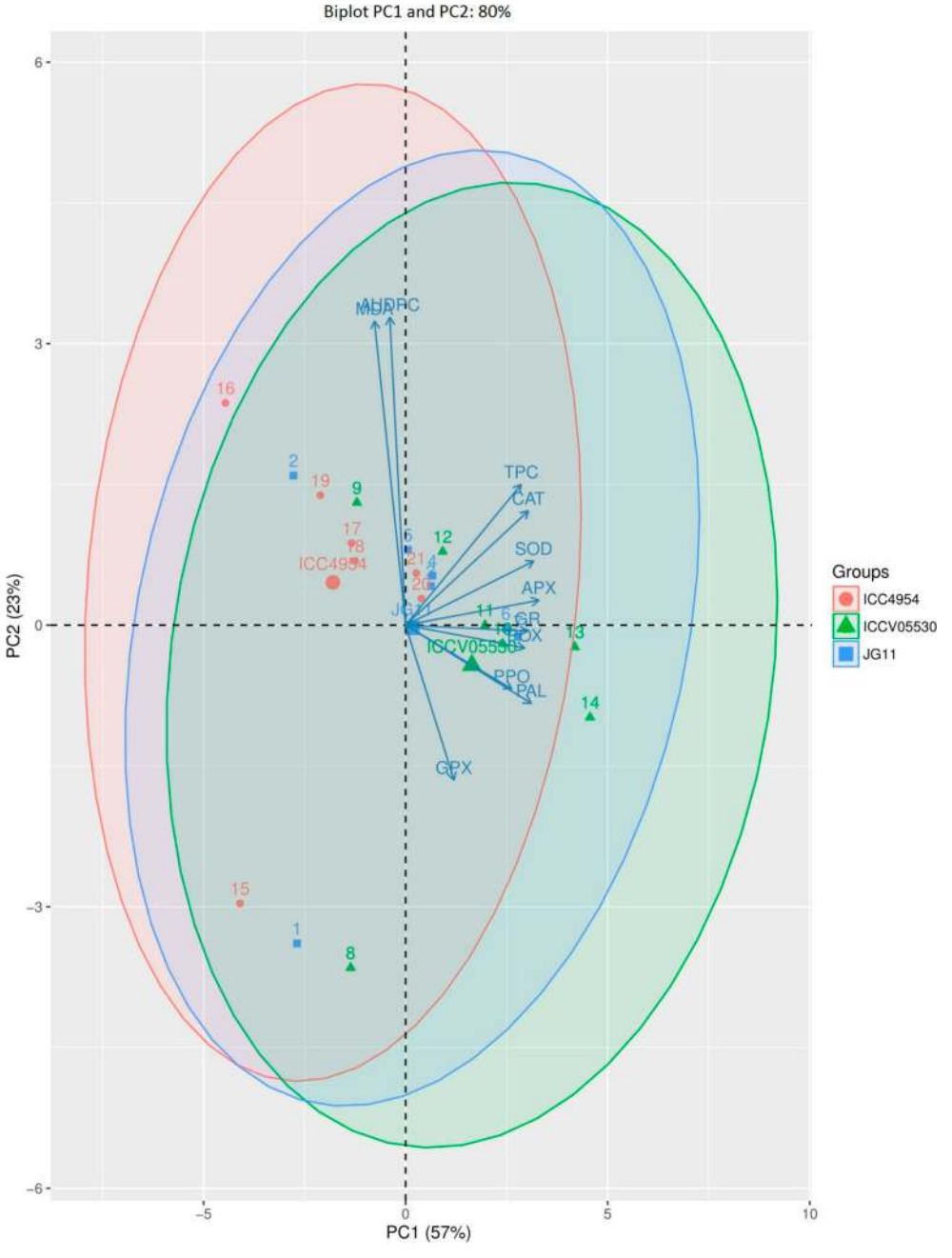

Figure 4. Principal component analysis on evaluation of all the studied antioxidant parameters in 3 chickpea genotypes during anti- *B. cinerea* study. 1, 8, 15 – Control; 2, 9, 16 – Disease control (*Bc* challenged); 3, 10, 17 – *Streptomyces* sp., ATIRS43 treated; 4, 11, 18 – *Streptomyces* sp., ATIRS-65 treated; 5, 12, 19 – *Streptomyces* sp., ARRS-10 treated; 6, 13, 20 – Consortium of ATIRS43 + ATIRS65 + ARRS10 treated; 7, 14, 21 – *T. harzianum* treated.

Table 5. Pearson correlation coefficients among the tested antioxidant parameters of 3 chickpea genotypes towards B. cinerea over the treatment with actinobacteria.

Antioxidant parameters	MDA	SOD	CAT	APX	GPX	GR	PAL	PPO	TPC
MDA	1								
SOD	−0.044^{NS}	1							
CAT	0.089^{NS}	.916**	1						
APX	−0.159^{NS}	.830**	.867**	1					
GPX	−0.371^{NS}	0.284^{NS}	0.091^{NS}	0.166^{NS}	1				
GR	−0.192^{NS}	.677**	.685**	.938**	0.194^{NS}	1			
PAL	−.437*	.788**	.758**	.838**	.481*	.734**	1		
PPO	−0.303^{NS}	.572**	.507*	.750**	0.283	.795**	.628**	1	
TPC	0.205^{NS}	.932**	.964**	.770**	0.173^{NS}	.573**	.709**	.442*	1

*Correlation is significant at $p < 0.05$; **Correlation is significant at $p < 0.01$; ^{NS} – Non significant.

ANOVA on antioxidant parameters confirms the above statements (Table 6). Among them, TPC, CAT and GPX were found to be non-significant between the genotypes and the remaining was found to be significant. Since antioxidant profile varies as genotype varies, the profiling is done in three different resistance spectrums. The highest significance ($p < 0.001$) on all the tested antioxidants (except GR) indicates the induction of antioxidants as a defense response towards microbial exposure. Time also has significance on antioxidants (except GPX). Highest significance on genotype and treatment interaction indicates microbial influence on antioxidants induction irrespective of the genotypes and its resistance. However, genotype and time interaction was significant only for GPX, GR and PPO.

Lignification

Actinobacteria influence on lignin deposition in chickpea stem sections of un-inoculated control, disease control and consortium treated plants were shown in Figure 5 (i, ii and iii). Faint pink colouration on the interfacial cambium (IFC) cells, phloem and xylem fibers of control plants indicates the normal lignification (Figure 5 (i, ii, iii and Control a–c)); whereas an intense pink to red colouration observed in *B. cinerea* challenged and consortium treated stem sections indicates higher lignin deposition (Figure 5 (i, ii, iii and Disease Control a–c & Consortium a–c)). This explains the role of microbes in influencing cell-wall components. Among the chickpea genotypes, JG11 and ICCV05530 showed similar lignification pattern; whereas ICC4954 has lower lignin depositions.

Discussion

Increased regulation of chemical pesticides, rapid evolution of pesticide resistance in insect pests and plant pathogens and associated yield loss has necessitated sustainable and acceptable pest management methods with the major focus on BCA especially plant-associated microorganisms (DiTomaso et al., 2017). Hence, we studied chickpea associated rhizospheric actinobacteria in inducing plant growth and host-plant resistance against *B. cinerea*, the causal organism of BGM disease in chickpea.

Of the 257 actinomycete isolates obtained from the rhizosphere soils of chickpea, only 10 (3.9%) were found to have acceptable level (>50% inhibition) of antagonistic potential against *B. cinerea*. The three most potential isolates, ATIRS43, ATIRS65 and ARRS10, that

Table 6. Analysis of variance (ANOVA) of antioxidant parameters in 3 chickpea genotypes.

		Mean square								
Source of variation	Df	MDA	SOD	CAT	APX	GPX	GR	PAL	PPO	TPC
Genotypes	2	130.64*	297.32**	13453^{NS}	4678***	15.29^{NS}	3357.96***	6.163**	3.048***	91.4^{NS}
Treatment	6	348.123***	492.58***	55130.6***	1793.5***	75.024***	316.1*	11.4503***	0.6316***	1649.141***
Time	4	5058.99***	2355.97***	185061***	11435***	19.68^{NS}	1042.9***	13.341***	4.0926***	7983***
Genotype × Treatment	12	3.4134***	1.8136***	161.54***	44.669***	9.9335***	72.0206***	0.10385***	0.060415***	4.1442***
Genotypes × Time	8	88.47^{NS}	20.42^{NS}	3063^{NS}	175.1^{NS}	56.62***	175.67***	0.694^{NS}	0.5221***	318.7^{NS}

*Significant at $p < 0.05$; **Significant at $p < 0.01$; ***Significant at $p < 0.001$; ^{NS} – Non significant.

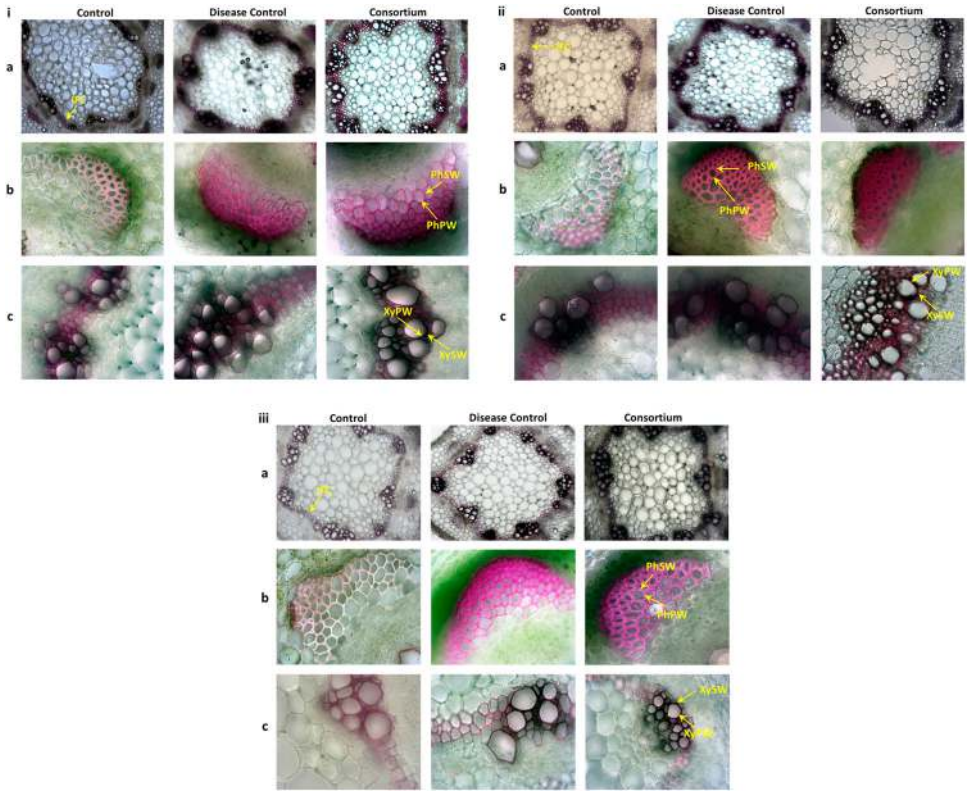

Figure 5. Representative photomicrographs of phloroglucinol-HCL stained stem sections of chickpea JG11 (i), ICCV05530 (ii) and ICC4954 (iii) towards microbial treatment. (a) – Cross section of chickpea stem (10×); (b) – Phloem of chickpea stem cross section (40×); (c) – Xylem of chickpea stem cross section (40×). IFC – Interfacial Cambium Cells, PhPW – Phloem Primary Wall, PhSW – Phloem Secondary Wall, XyPW – Xylem Primary Wall, XySW – Xylem Secondary Wall.

registered more than 67% inhibitory activity in dual culture assay, were further studied. Li et al. (2012) observed 100% inhibition of *B. cinerea* by *Streptomyces globisporus* JK-1. You et al. (2016) stressed the importance of screening BCA agents against *B. cinerea* at 15–20° C. Henceforth, in the present study, the dual culture assay was performed at 19°C in order to mimic *B. cinerea* growing conditions, which in turn indicate the suitability of selected strains as BCA for *B. cinerea*.

In the current study, the selected *Streptomyces* strains were found to produce HCN and/or ammonia, the key inhibitory volatiles and diffusible compounds; siderophores, the low-molecular-weight, high affinity iron trapping molecules and the lytic enzymes targeting pathogen's cell-wall (Table 1). *S. globisporus* JK-1 was reported to produce an array of volatiles including alcohols, acids, alkenes, alkanes, esters and ketones with inhibitory activity on conidial germination and appressorium formation of *B. cinerea* on tomato (Li et al., 2012). Report of Złoch, Thiem, Gadzała-Kopciuch, and Hrynkiewicz (2016) observed that, *Streptomyces* is the highest producer of siderophore in the rhizospheric community of *Betula pendula* and *Alnus glutinosa*. *Streptomyces* sp., 9X166 with β-1,3-glucanase was shown to have antagonistic activity against *Phytophthora* (Sakdapetsiri,

Fukuta, Aramsirirujiwet, Shirasaka, & Kitpreechavanich, 2016). It is concluded that HCN/ammonia/siderophore and or lytic enzymes production could be one of the mechanisms for the selected *Streptomyces* strain's inhibitory activity against *B. cinerea*.

In the current study, the three selected *Streptomyces* strains, ATIRS43, ATIRS65 and ARRS10, produced IAA (with the highest activity on ATIRS43; 62 μg ml^{-1}), solubilise phosphate, potassium (except ARRS10), and one or other forms of zinc. ATIRS43 was also found to produce ACC deaminase (Table 2). *Streptomyces* strains from chickpea, wheat and tomato rhizosphere were reported to produce IAA and help in PGP traits (Anwar, Ali, & Sajid, 2016; Gopalakrishnan et al., 2014). Correlating results for P, K and Zn solubilisation were reported on *Micromonospora aurantiaca* and *Streptomyces griseus* for P (Hamdali, Hafidi, Virolle, & Ouhdouch, 2008), *Micrococcus foliorum* for K (Zhang & Kong, 2014) and *Streptomyces* sp. for Zn solubilisation (Thampi & Bhai, 2017). Actinobacterial members belong to the genus *Rhodococcus*, *Nocardia* and *Streptomyces* were reported to produce ACC deaminase (Nascimento, Rossi, & Glick, 2016).

The three selected *Streptomyces* strains were demonstrated to have *in planta* growth-promoting effects on chickpea variety JG11 by consortium followed by single inoculations with ATIRS65. Though there were some significant growth responses on 45 day observations with ATIRS43 and ARRS10, these were found to be insignificant at harvest. This is in line with Couillerot et al. (2013) who observed significant increases on shoot dry weights of maize with two (*Pseudomonas* + *Glomus*) and three component consortium (*Pseudomonas* + *Glomus* + *Azospirillum*) on 10^{th} and 21^{st} day of samplings over the un-inoculated control but not on 30^{th} day. It is understood that, co-inoculation of PGP strains might offer complementary functions for enhanced host crop nutrition and yield; PCA analysis confirms this magnitude as control > single inoculation > consortium. IAA produced by rhizospheric bacteria increases number of adventitious roots which in turn promotes uptake of greater volume of nutrients and water by plants. Also increased plant root exudates benefits bacterial nutrition resulting in good plant-microbe mutualistic relationships (El-Tarabily, 2008). In the present study, PCA analysis showed increased root growth by ATIRS65 (with lower IAA production; Table 2) and shoot growth by ATIRS43 (with higher IAA production; Table 2). This is in line with Long, Schmidt, Baldwin, and Ausubel (2008) who observed higher root length with lower bacterial IAA and *vice versa* in *Solanum nigrum* seedlings. Positive correlations observed in the present study may be corroborated with similar positive correlations observed for IAA, siderophore, HCN, ammonia and ACC deaminase of PGP microbes such as *Microbacterium*, *Rhizobium*, *Pseudomonas* and *Bacillus* on tomato seedlings (Karthik, Pushpakanth, Krishnamoorthy, & Senthilkumar, 2017).

Co-inoculation of beneficial rhizospheric bacteria with nodulating rhizobia is known to promote nodulation, nitrogen fixation and crop productivity (Egamberdieva et al., 2010). We also found enhanced nodulation and nitrogenase activity in chickpea ICCV10 and JG11 by actinobacterial co-inoculation with *M. ciceri* than *M. ciceri* alone which confirms the helper effect. Similar observations were seen by co-inoculation of *Streptomyces* MM40, *Actinoplanes* ME3, and *Micromonospora* MM18 with *Mesorhizobium loti* in *Lotus tenuis* (Solans, Ruiz, & Wall, 2015).

BCA not only affect the pathogen but also induce host plants immune system to resist pathogens (Glick, 2012). Microbial treatments in the present study have significantly reduced the disease progression by 28–47% over the disease control groups, in which

consortium and *T. harzianum* showed comparably equal and higher effectiveness irrespective of the chickpea genotypes than single inoculations. Similar observation was also reported for *Trichoderma* consortium on strawberry BGM (Freeman et al., 2004).

Being a necrotroph, *B. cinerea* employs various virulence mechanisms to induce host's cell death so as to get nutrition for its development. Induction of reactive oxygen intermediates is one such key mechanism (Govrin & Levine, 2000). In the present study, the extent of oxidative stress due to *B. cinerea* is determined through MDA, an oxidised product of unsaturated fatty acids. *B. cinerea* challenge has increased MDA content irrespective of the chickpea genotypes. The highest content of MDA was observed in ICC4954 (susceptible to BGM) than in ICCV05530 (moderately resistant to BGM) genotype. This result complies with Mutlu, Atıcı, Nalbantoğlu, and Mete (2016) who noticed the same trend on cold sensitive and resistant barley cultivars. Reduced MDA content in actinobacteria and *T. harzianum* treated plants indicates the nearly balanced oxidative status. Identical effects were noticed with *Streptomyces hygroscopicus* against *Fusarium oxysporum* on strawberry (Shen et al., 2016).

During the pathogenesis process, NADPH oxidase of *B. cinerea* contributes for O_2- as a virulence factor and initiates a cascade of oxidative burst (Choquer et al., 2007). Cytochemical studies identified O_2- in hyphal tips and H_2O_2 in penetrated host cell-wall, germinating spores and in infection cushions. Findings of the current study shows induction of SOD against both *B. cinerea* challenge and microbial treatments as a first line defense tool irrespective of the chickpea genotypes; however, the extent of SOD induction was high in the later groups. This gives a clear indication that, priming of plants with beneficial bacteria before the pathogen exposure enhances the resistance levels (Beckers & Conrath, 2007). CAT, the first line defense associated enzyme works sequentially with SOD to scavenge H_2O_2, as dismutation of O_2- leads to H_2O_2 generation. Singh et al. (2013) documented induction of SOD and CAT on chickpea by rhizospheric microbial consortium consists of fluorescent *Pseudomonas*, *Trichoderma* and *Rhizobium* against *Sclerotium rolfsii*.

The next crucial enzymes are APX, GPX and GR which involve in complete neutralisation of H_2O_2 (Ushimaru, Shibasaka, & Tsuji, 1992). The combined activity of APX and GR were proven in the present study by its significant positive correlation. Demonstrations with significant correlation of SOD, GPX and APX in resistant groundnut varieties than in susceptible varieties by *Trichoderma viride* against the collar rot confers further support to this study (Gajera, Katakpara, Patel, & Golakiya, 2016).

Among the plants' alarming system related to induced-systemic resistance, induction of phenylpropanoid pathway (PPP) has the core value. This cascade is initiated by PAL and later by PPO, for the synthesis of phenolics and related compounds. These phenolics are synthesised on recognition of microbe associated molecular patterns (MAMPs) by the plant pattern recognition receptors (Bittel & Robatzek, 2007). In the present study, phenolics content were increased by the exposure of both pathogen and actinobacteria. However, higher contents were noticed in selected actinobacterial and *Trichoderma* treatments suggesting that receptors for chickpea associated phenolics responds better to beneficial actinobacterial receptors. A great line of evidence were available for increased PAL, PPO and TPC by beneficial microbes such as *Pseudomonas*, *Trichoderma* and *Rhizobium* against *S. rolfsii* in chickpea (Singh et al., 2013). In the present study, genotypes ICCV05530 and JG11 produced comparably equal and higher phenolics than ICC4954

(susceptible to BGM). Similar results were observed by Mitter, Grewal, and Pal (1997). They observed higher phenolics in BGM resistant chickpea genotype ICC1069 than in sensitive genotype BGM408.

In the present study, qualitative and quantitative changes of phenolics in chickpea during its interaction with microbes have been documented with moderately resistant (to BGM) genotype ICCV05530. Presence of ferrulic acid, sinapic acid, chlorogenic acid and cinnamic acid in microbial treatments indicate the induction of PPP. Similar results were reported in onion towards *Botrytis allii* infection with induced hydroxycinnamic acid derivatives such as feruloyl-3′-methoxytyramine, feruloyltyramine, and pcoumaroyltyramine (McLusky et al., 1999). Occurrence of higher fold change for hydroxycinnamic acid derivatives among the identified resistance related metabolites against *Phytophthora infestans* in potato (Yogendra et al., 2014) further supports this phenomenon.

Lignin, a branched phenylpropanoid polymer of plant cell-wall, often increases as defense responses to stress conditions, because lignification decreases cell-wall plasticity and cell growth (Voxeur, Wang, & Sibout, 2015). In the present study, changes in lignification were documented over treatments and also genotypes. Similarly, increased lignification on chickpea towards *S. rolfsii* infection (Singh et al., 2013) and on alfalfa towards *Rhizobium meliloti* infection (Zhang et al., 2016) had been demonstrated. The microscopic images on lignification have a clear line with our phenolic profiling as follows; intense lignification observed on *B. cinerea* challenged and consortium treated stem sections might have appeared due to the induction of hydroxycinnamic acid derivatives and flavonoids of the same group over control group. Solid evidences are available in the metabolo-proteomic study on wheat's resistance mechanism against *Fusarium graminearum* (Gunnaiah et al., 2012). Laser scanning confocal microscopic images of rachis sections shows hydroxy cinnamic acid amides and flavonoids in secondary cell-wall thickening and thereby restricting the pathogen entry. Further analysis on LC-hybrid MS identified eight hydroxy cinnamic acid amides and five flavonoids. Observations in the study indicate that the pathogen protection at basic level and more molecular approaches might substantiate the factual.

Conclusions

The selected chickpea rhizospheric *Streptomyces* sp. with plant growth-promoting ability and anti-*B. cinerea* activity was able to induce crop growth and yield components; and also offered protection towards *B. cinerea* through induced host-plant resistance under controlled conditions as a consortium than as individual isolates. Further, the present results sufficiently convinced the role of antioxidants in host-plant resistance and suggest candidate isolates for further molecular investigations. Better understanding on all other mechanisms and field studies will further facilitate their application not only for biocontrol of *B. cinerea* in chickpea but also for control of BGM in fruits and vegetables.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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