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Evaluation of *Streptomyces* spp. for their plant growth-promotion traits in rice

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Abstract: Five strains of *Streptomyces* (CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27) were earlier reported to have potential for charcoal rot control and plant growth promotion (PGP) in sorghum. In this study, those five *Streptomyces* strains were characterized for their enzymatic activities and evaluated for their PGP capabilities on rice. All the *Streptomyces* were able to produce lipase, β-1,3-glucanase, grew in NaCl (up to 8%), at pH 5−13, temperatures 20−40^{o}C and were resistant to ampicillin, sensitive to nalidixic acid and highly sensitive to chloramphenicol, kanamycin, streptomycin and tetracycline. They were highly tolerant to fungicide bavistin, whereas highly sensitive to benlate, benomyl and radonil. When evaluated on rice in the field, the *Streptomyces* significantly enhanced tillers, panicles, stover yield, grain yield, dry matter, root length, volume and dry weight over the control. In the rhizosphere at harvest, microbial biomass carbon and nitrogen, dehydrogenase activity, total N, available P and % organic carbon were also found significantly higher in *Streptomyces* treated plots over the control. This study further confirms that the selected *Streptomyces* have PGP activities.

Keywords: plant growth promotion, rice, *Streptomyces*, field evaluation.

Introduction

The use of plant growth-promoting (PGP) microorganisms for sustainable agriculture has increased tremendously in many parts of the world as significant increases in the growth and yield of agriculturally important crops have been widely reported (Biswas et al. 2000; Asghar et al. 2002; Vessey 2003; Figueiredo et al. 2008; Gopalakrishnan et al. 2012a, b). PGP microorganisms may facilitate plant growth either by direct stimulation (such as fixed nitrogen, soluble phosphate, iron chelators and phytohormones) or by indirect stimulation (such as inhibiting phytopathogens). Strains of *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp. and *Streptomyces* spp. were found effective in helping the plants not only to mobilize and acquire nutrients (Perner et al. 2006) but also to control phytopathogens (Postma et al. 2003; Khan et al. 2004; Gopalakrishnan et al. 2011a, b, c; Gopalakrishnan et al. 2012a, b). *Streptomyces* are a group of Gram-positive bacteria, with high G + C content belonging to the order Actinomycetales, which form branched mycelia and hence sometimes been classified as fungi imperfecti. Plant growth promotion potential of *Streptomyces* was reported on bean (Nassar et al. 2003), tomato (El-Tarabily 2008), pea (Tokala et al. 2002), wheat (Sadeghi et al. 2012) and rice (Gopalakrishnan et al. 2012a, b). *Streptomyces* promote plant growth either by producing indole-3-acetic acid (Aldesuquy et al. 1998) or siderophores (Tokala et al. 2002). *Streptomyces* has also been extensively studied and used for biocontrol of soil-borne fungal pathogens (Mahadevan and Crawford 1997; Trejo-Estrada et al. 1998; Macagnan et al. 2008.

Earlier, we reported a set of eight *Streptomyces* strains (CAI-21, CAI-26, MMA-32, CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27) isolated from herbal vermicompost, with the potential for PGP and control of charcoal-rot disease, caused by *Macrophomina phaseolina* (Tassi) Goid., in sorghum (Gopalakrishnan et al. 2011b). The first three of the eight *Streptomyces* strains (CAI-21, CAI-26 and MMA-32) were also reported to have potential for PGP in rice (Gopalakrishnan et al. 2012a). The objective of this study was to further characterize the remaining five *Streptomyces* strains (CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27) for their enzymatic activities (cellulase, lipase and β-1, 3-glucanase), physiological traits (salinity, temperature, pH and resistance to antibiotics and fungicides) and evaluate, under field conditions, their PGP traits in rice grown using the system of rice intensification (SRI; Uphoff 2001; Kumar et al. 2010) method.

Materials and methods

***Streptomyces* strains**

Five strains of *Streptomyces* isolated from herbal vermicompost, CAI-17 (*Streptomyces* spp. from *Chrysanthemum morifolium* foliage compost; NCBI Accession number: JQ682619), CAI-68 (*Streptomyces* spp. from *Nerium indicum* foliage compost; NCBI Accession number: JQ682622), CAI-78 (*Streptomyces* spp. from *Parthenium hysterophorus* foliage compost; NCBI Accession number: JQ682623), KAI-26 (*Streptomyces* spp. from rice straw compost; NCBI Accession number: JQ682624) and KAI-27 (*Streptomyces* spp. from rice straw compost; NCBI Accession number: JQ682625), reported earlier by us as potential for PGP and biocontrol traits in sorghum (Gopalakrishnan et al. 2011b), were further studied.

Evaluation of *Streptomyces* for their enzymatic activities

Production of cellulase and lipase

The standardized protocols of Hendricks et al. (1995) were used to evaluate the cellulase production for all the five strains of *Streptomyces* (CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27). Lipase production was done as per the methodologies of Bhattarcharya et al. (2009). In brief, the *Streptomyces* were streaked on Tween 80 agar and incubated at 28 °C for five days. The plates were observed for halo zone around the *Streptomyces* colonies which indicate the presence of lipase. Treatments were replicated 3 times and the experiment was conducted 3 times.

Observations of the five *Streptomyces* strains to cellulase and lipase were recorded on a 0−5 rating scale as follows: 0 = no change; 1 = positive; 2 = halo zone of 1−3 mm; 3 = halo zone of 4−6 mm; 4 = halo zone of 7−9 mm and 5 = halo zone of 10 and above.

Production of β-1,3-glucanase

It was done as per the protocols of Singh et al. (1999). *Streptomyces* strains were cultured individually in Tryptic soy broth, supplemented with 1% (weight/volume) colloidal chitin, at 28 ^{o}C for four days. Treatments were replicated 3 times and the experiment was conducted 3 times. At the end of the incubation, the cultures were centrifuged at 10,000*g* for 12 min and the supernatants collected. One ml of the culture filtrate was allowed to react with 0.1 ml of laminarin solution (2%, weight/volume) in 0.2 M acetate buffer (pH 5.4) at 40 ^{o}C for 1 h. The reaction was stopped by adding 3 ml of dinitrosalicylic acid to the mixture and the color of the end product was developed by boiling for 10 min. At the end of the incubation, development of dark red color indicated the presence of β-1,3-glucanase, and the concentration of the reducing sugar was determined by

measuring the absorbance at 530 nm using a spectrophotometer. Calibration standards were prepared using glucose at 0−1 mg mL^{-1} at the interval of 0.2 mg mL^{-1}. One unit of β-1,3-glucanase activity was defined as the amount of enzyme that liberated 1 µmol of glucose $hour^{-1}$ at defined conditions.

Evaluation of *Streptomyces* for their physiological traits

Salinity, pH, temperature and resistance to antibiotics and fungicides

The five strains of *Streptomyces* (CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27) were streaked on Bennett's agar with various concentrations of NaCl ranging from 0% to 12% at an interval of 2%. The plates were incubated at 28 ^{o}C for five days and the intensity of growth was measured at the end of incubation. For pH, the five strains were streaked on Bennett's agar, adjusted to pH 5, 7, 9, 11 and 13, and incubated for five days at 28 ^{o}C, whereas for pH 3, the Bennett's broth was inoculated, and at the end of the five-day incubation the intensity of growth was measured at 600 nm in a spectrophotometer. For temperature, the *Streptomyces* were streaked on Bennett's agar and incubated at 20, 30 and 40 ^{o}C for five days, while for 50 ^{o}C, the Bennett's broth was inoculated, and at the end of the five-day incubation, the intensity of growth was measured at 600 nm in a spectrophotometer. Treatments were replicated 3 times and the experiment was conducted 3 times.

A total of six antibiotics namely ampicillin, chloramphenicol, kanamycin, nalidixic acid, streptomycin and tetracycline were studied for their resistance pattern against the five *Streptomyces* as per the standardized protocols of Gopalakrishnan et al. (2012a). The five strains of *Streptomyces* were also evaluated for their tolerance to fungicides at field application level. The fungicides studied include Thiram (dimethylcarbamothioylsulfanyl *N*, *N*-dimethylcarbamodithioate), Bavistin (carbendazim 50%; methyl benzimidazol-2-ylcarbamate), Benlate (benomyl 50%; methyl [1-[(butylamino) carbonyl]-1H-benzimidazol-2-yl] carbamate), Captan (captan 50%; *N*-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide), Benomyl (methyl [1-[(butylamino)carbonyl]-1H-benzimidazol-2-yl] carbamate) and Radonil (*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester) at field application levels of 3000, 2500, 4000, 3000, 3000 and 3000 ppm concentrations, respectively. The required quantities of antibiotics/fungicides were dissolved in sterilized Milli-Q water and mixed into Bennett's agar just before pouring into the Petri plates (when the temperature of the media was about 50 ^{o}C). The plates were incubated at 28 ^{o}C for five days and the intensity of growth was measured at the end of incubation. There were three replications for each test and the experiment was done thrice.

Observations of the five *Streptomyces* strains to salinity, pH, temperature and fungicide tolerance were recorded as follows: 0 = no growth; 1 = slight growth; 2 = medium growth and 3 = good growth.

Evaluation of *Streptomyces* for PGP potential on rice under field conditions

The experiment was conducted in 2011−2012 (post rainy season) at ICRISAT, Patancheru, Andhra Pradesh, India) with a medium duration rice variety, Sampada (135 days), which normally yields 6.5−7.0 t ha^{-1}. Soils at the experimental site are classified as sandy loam in texture (55% sand, 17% silt and 28% clay) with alkaline pH of 8.5−9.4 and organic carbon content of 0.76−1.27%. The mineral content of the rhizosphere (top 15cm layer) was as follows: available nitrogen 292kg ha^{-1}, available phosphorus 26.8kg ha^{-1} and available potassium 527kg ha^{-1}. The experiment was laid out in a randomized complete block design (RCBD) with three replicates and subplot sizes of 10 × 7.5 m. Rice was grown by the system of rice intensification (SRI) method proposed by the Central Rice Research Institute (http://crri.nic.in), Cuttack, Orissa, India. The five strains of *Streptomyces* (CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27) were grown on a starch casein broth at 28 ^{o}C for five days and further evaluated for their PGP traits. The control contained no *Streptomyces* strains.

The field experiment was conducted as described previously (Gopalakrishnan et al. 2012a). In brief, the 12-day-old single seedlings were uprooted from the nursery, their roots dipped in the respective *Streptomyces* spp. broth (containing 10^8 CFU ml^{-1}) for 50 min and transplanted on 27th December 2011 at a row-to row spacing of 25 cm and a plant-to-plant spacing of 25 cm. The *Streptomyces* (1000ml; 10^8 CFU mL^{-1}) were applied once in 15 days until the flowering stage along with the irrigation. Irrigation was done as recommended for the SRI method, i.e. the alternate wetting and drying method. Weeding was done four times by cono-weeder to incorporate weeds into the soil at 10, 20, 30 and 40 days after transplanting. No serious insect-pests or phytopathogens attack were observed during the cropping period. The crop was harvested manually on 23rd May 2012 and observed for plant height (cm), total panicles ($plant^{-1}$), panicle length (cm), filled grain number and weight (g), total tillers (m^{-2}), stover yield (g m^{-2}), grain yield (g m^{-2}) and total dry matter (g m^{-2}). Root samples were collected from 0 to 30 cm soil profile and analyzed for root length (mm^{-2}; EPSON expression 1640×, Japan), volume (cm^3m^{-2}) and dry weight (gm^{-2}) dried in an oven at 70 ^{o}C for 48 h. Soil samples were collected from 0 to 15 cm soil profile, at harvest, and analyzed for soil chemistry (total nitrogen [ppm], available phosphorous [ppm] and % organic carbon as per the protocols of Novozamsky et al. [1983], Olsen and Sommers [1982] and Nelson and Sommers [1982], respectively) and biological analysis (microbial biomass carbon [µg g−1 soil] by fumigation method, microbial biomass nitrogen [µg g−1 soil] by Kjeldahl distillation method and

dehydrogenase activity [µg TPF g^{-1} soil 24 h^{-1}] by Triphenyl formazan production method as per the protocols of Anderson and Domsch [1989], Brooks et al. [1985] and Casida [1977], respectively).

Statistical analysis

Data were analyzed by using Analysis of Variance (ANOVA) technique, by SAS GLM (General Linear Model) procedure (SAS Inst. 2002-08, SAS V9.3) considering isolates and replication as fixed in RCBD. Depth-wise ANOVA was performed for the traits root length, volume and dry weight. Isolate means were tested for significance and compared using Fisher's protected least significant difference (LSD).

Results

Evaluation of *Streptomyces* for their enzymatic activities and physiological traits

When the five *M. phaseolina* antagonistic *Streptomyces* were evaluated for their enzymatic activities, all strains produced β-1,3-glucanase while *Streptomyces* strains CAI-17, CAI-68, CAI-78 and KAI-26 were able to produce cellulase (Table 1). *Streptomyces* strains CAI-17, CAI-78 and KAI-26 were able to grow up to 10% NaCl and none grew at 12% of NaCl conditions. *Streptomyces* grown under a gradient of pH indicated that none of the isolates grew in pH 3 and all of them grew well from pH 7 to pH 13. A pH of 5 was discriminatory for the strains, the strain CAI-17 showed medium growth, while others exhibited poor growth. Temperatures between 20 and 40 ^{o}C were found optimum for growth of all *Streptomyces* strains, whereas none of them grew at 50 ^{o}C. With regard to antibiotic resistance pattern studies, *Streptomyces* strains CAI-17, CAI-78, KAI-26 and KAI-27 were found highly resistant to ampicillin (>1800 ppm), while all strains were found sensitive to nalidixic acid (<50 ppm) and highly sensitive to chloramphenicol, kanamycin, streptomycin and tetracycline (<25 ppm). The *Streptomyces*, at field application level, were found highly tolerant to fungicide bavistin, slightly tolerant to thiram (except KAI-27) and captan (except CAI-78) but highly sensitive to benlate, benomyl and radonil (Table 2).

Evaluation of *Streptomyces* for PGP potential on rice under field conditions

Under field conditions, when the five *M. phaseolina* antagonistic *Streptomyces* were evaluated for their PGP potential against an untreated control, the *Streptomyces*-treated plots significantly enhanced plant height, total panicles, filled grain numbers and weight, total tillers (11−25%), stover yield (16−91%), grain yield (1−25%) and total dry matter (14−58%) over the untreated control (Tables 3 and 4). In addition, the plots treated

with *Streptomyces* significantly enhanced the root development, at both 0−15 and 15−30 cm depths, including the root length (16−34%), the root volume (29−53%) and the root dry weight (14−58%) over the control (Table 5). Of the five *Streptomyces* studied, CAI-78 enhanced the yield parameters (including stover yield, grain yield and total dry matter) and root development (including root length, root volume and root dry weight).

The soil biological activities in the top 15 cm rhizosphere soils, at harvest, including microbial biomass carbon, microbial biomass nitrogen and dehydrogenase activity were found significantly enhanced (23−48%, 7−321% and 14−278%, respectively) in the *Streptomyces*-inoculated plots over the untreated control. Further, in the *Streptomyces*-inoculated plots the soil mineral nutrients such as total N, available P and % organic carbon were also found significantly higher than the untreated control (8−82%, 13−44% and 17−39%, respectively) in the top 15 cm rhizosphere at rice harvest (Table 6).

Discussion

Five *Streptomyces* strains reported in this study (CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27) were known to be good biocontrol agents of sorghum charcoal rot disease as well as sorghum plant growth promoters by producing siderophore and indole acetic acid (IAA) (Gopalakrishnan et al. 2011b). Siderophores produced by bacteria bind Fe^{3+} from the environment and make it available to the plants in addition to its own growth (Wang et al. 1993), whereas IAA-producing microorganisms are known to stimulate growth of the plants, particularly roots (Patten and Glick 2002); these two traits can be exploited by the host plants for their PGP. Therefore, in the present study, the five *Streptomyces* strains were characterized for their enzymatic activities, physiological traits and further evaluated for their PGP traits under field conditions on rice grown by SRI methods.

In the present investigation, all the five *Streptomyces* strains were able to produce lipase and β-1,3-glucanase and *Streptomyces* strains CAI-17, CAI-68, CAI-78 and KAI-26 produced cellulase. Cellulose and lipid present abundantly in the plant biomass can be degraded by enzymes such as cellulase and lipase (Lynd et al. 2002). The cell wall of higher pathogenic fungi, such as *Fusarium oxysporum*, is composed of layers of β-1,3-glucan and lysis of this by β-1,3-glucanase-producing microbe leads to leakage of cell contents and collapse of the pathogenic fungi (Singh et al. 1999; Macagnan et al. 2008). Hence, microorganisms having these traits can be exploited for degradation of organic residues and/ or biological control of plant pathogens.

The five *Streptomyces* strains antagonistic to *M. phaseolina* were able to grow in NaCl up to 8%, pH values between 5 and 13, temperatures between 20 and 40 ^{o}C and found highly resistant to ampicillin and

tolerant to fungicide bavistin. The ability of *Streptomyces* to tolerate abiotic stresses including salinity, pH, and temperature and antibiotics and fungicide tolerance are widely reported (Waksman 1959, Gopalakrishnan et al. 2012a, b; Sadeghi et al. 2012). Consequently, it can be stated that these strains have the capability to survive in harsh environments and can be used in the integrated disease management programs.

In field, the five *Streptomyces* strains significantly enhanced morphological and yield traits of rice including plant height, total panicles, filled grain numbers and weight, total tillers, root length and volume, root dry weight, stover yield, grain yield and total dry matter over the un-treated control. The efficacy of *Streptomyces* strains for PGP is extensively reported (Nassar et al. 2003; El-Tarabily 2008; Gopalakrishnan et al. 2012a, b). The soil biological activities and mineral nutrients (including microbial biomass carbon and nitrogen, dehydrogenase, total N, available P and % organic carbon) in the *Streptomyces*-treated rice plots, at harvest, were also found significantly higher over the untreated control plots. The mechanism by which the *Streptomyces* strains enhanced morphological and yield traits of rice could be attributed to their enzymatic activities such as IAA and siderophore (direct stimulation of PGP) and/or chitinase, protease, hydrocyanic acid, cellulase, lipase and β-1,3-glucanase production capabilities (indirect stimulation of PGP; Gopalakrishnan et al. 2011b). The influence of microorganisms on PGP including the root development of the plants has been reported by Birkhofer et al. (2008), Uphoff et al. (2009) and Gopalakrishnan et al. (2011a, b, 2012a, b). Though the SRI method of rice cultivation supports the growth of PGP microbes (including phosphate solubilizing bacteria, diazotrophs, *Azospirillium* and *Azotobacter*) and microbial enzyme activities (Turner and Haygarth 2001; Gayathry 2002) such enhanced activities were found, in the present investigation, only in the *Streptomyces*-inoculated treatments. Hence, it is concluded that the five *Streptomyces* strains were able to survive in the rice rhizosphere and enhance soil health.

In this study, although roots were not inspected for colonization, the data on root morphology (including root volume, length and dry weight), soil biological (microbial biomass carbon, nitrogen and dehydrogenase) and chemical activities (total N, available P and % organic carbon) in the rhizosphere (0−15 cm) strongly suggest that the five *Streptomyces* strains had multiplied and colonized the roots of rice plants. Therefore it is concluded that the five *Streptomyces* strains used in this study were apparently well adapted not only in the sorghum rhizosphere (Gopalakrishnan et al. 2011b) but also in the rice rhizosphere, in the present investigation, where they promoted plant growth. Further, the five *Streptomyces* strains could also be used as biocontrol agents against charcoal rot disease in sorghum.

The five *Streptomyces* strains used in this investigation were apparently containing broad range of PGP and antifungal traits and demonstrate multiple mechanisms of actions indicating its broad spectrum activity. Broad spectrum PGP agents offer effective novel strategies not only for crop growth but also for controlling multiple pathogens and insect pests that attack crops. In addition to suppressing plant pathogens by secretion of antibiotics, some PGP microbes can also elicit induced systemic resistance (ISR) against a broad range of pathogens, insects and nematodes (Jetiyanon and Kloepper 2002; Ryu et al. 2007). Therefore, the five *Streptomyces* strains used in this investigation are likely to be the potential candidates for discovery of novel secondary metabolites for various PGP and biocontrol applications. Further, determinations of their usefulness in host plant resistance against range of pathogens and insect pests can assist in furthering the use of eco-friendly bio-pesticides and bio-fertilizers. The use of PGP microbes in developing such bio-products will probably be one of the important tactics of integrated pest, disease and nutrition management of the world in the near future.

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Table legends

Table 1. Enzymatic activities by the five *Streptomyces* strains.

The rating scales for cellulase and lipase were as follows: 0 = no change; 1 = positive; 2 = halo zone of 1−3 mm; 3 = halo zone of 4−6 mm; 4 = halo zone of 7−9 mm and 5 = halo zone of 10 and above.

*= One unit of β-1,3-glucanase is defined as the amount of enzymes causing the release of 1 µmol of glucose equivalent per hour under the conditions described in materials and methods.

Table 2. Effect of salinity, pH, temperature and antibiotics and fungicides tolerance on the growth of *Streptomyces strains*.

***= Statistically significant at 0.001, SE in parentheses is to compare means within same treatment. # Responses of the five actinomycetes to salinity, pH, temperature and fungicide tolerance were recorded as follows: 0 = No growth, 1 = Poor growth; 2 = Medium growth; 3 = Good growth.

Table 3. Effect of *Streptomyces* strains on the morphology of rice cultivation.

SE, standard error; LSD, least significance difference; CV, coefficient of variation; *, statistically significant at 0.01; **, statistically significant at 0.01; ***, statistically significant at 0.001.

Table 4. Effect of *Streptomyces* strains on the yield potential of rice cultivation.

SE, standard error; LSD, least significance difference; CV, coefficient of variance; *** statistically significant at 0.001.

Table 5. Effect of *Streptomyces* strains on the root development of rice, at harvest, of rice cultivation.

SE, standard error; CV, coefficient of variance; ***, statistically significant at 0.001; SE in parentheses are to compare means within the same treatment.

Table 6. Effect of *Streptomyces* strains on soil biological and chemical activities, at harvest, of rice cultivation.

N, nitrogen; P, phosphorous; ppm, parts per million; SE, standard error; LSD, least significance difference; CV, coefficient of variance; **, statistically significant at 0.01; ***, statistically significant at 0.001.

Table 1. Gopalakrishnan et al. 2013

Traits	CAI-17	CAI-68	CAI-78	KAI-26	KAI-27
Cellulase	3.3	4.0	4.0	3.3	0.0
Lipase	5.0	3.7	5.0	5.0	4.0
β-1,3-Glucanase (units*)	0.61	0.66	2.92	0.35	0.20

Table 2 Gopalakrishnan et al. 2013

Traits	CAI-17	CAI-68	CAI-78	KAI-26	KAI-27	Mean
Salinity [#]						
0	3	3	3	3	3	3
2	3	3	3	3	3	3
4	3	3	3	3	3	3
6	3	3	3	3	3	3
8	3	1	3	3	1	2
10	2	0	2	2	0	1
12	0	0	0	0	0	0
SE±			0.1(0.1) ***			0.06***
CV%			12			
pH [#]						
3	0	0	0	0	0	0
5	2	1	1	1	1	1
7	3	3	3	3	3	3
9	3	3	3	3	3	3
11	3	3	3	3	3	3
13	3	3	3	3	3	3
SE±			0.1(0.1) ***			0.03***
CV%			5			
Temperature (^{O}C) [#]						
20	3	3	3	3	3	3
30	3	3	3	3	3	3
40	3	3	3	3	3	3
50	0	0	0	0	0	0
SE±			0.003(0.003)***			0.002***
CV%			1			
Fungicide tolerance [#]						
Thiram @ 3000 (ppm)	1	1	1	1	0	1
Bavistin @ 2500 (ppm)	3	3	3	3	3	3
Benlate @ 4000 (ppm)	0	0	0	0	0	0
Captan @ 3000 (ppm)	2	1	0	1	1	1
Benemyl @ 3000 (ppm)	0	0	0	0	0	0
Radonil @ 3000 (ppm)	0	0	0	0	0	0
SE±			0.09(0.09)***			0.04***
CV%			18			
Antibiotics resistance pattern (ppm)						
Ampicillin	1800	100	1800	1800	1800	1460
Chloramphenicol	25	25	25	5	5	17
Kanamycin	50	5	5	4	4	14
Nalidixic Acid	50	50	50	50	50	50
Streptomycin	15	100	15	5	5	28
Tetracyline	2	15	1.5	9	9	7
SE±			0.5(0.5) ***			0.2***
CV%			1			

Table 3. Gopalakrishnan et al. 2013

Treatment	Plant height (cm)	Total panicles (plant^{-1})	Panicle length (cm)	Filled grain number	Filled grain weight (g)	Total tillers (m^{-2})
CAI-17	73	39	22	128	2.04	573
CAI-68	72	40	23	135	2.10	539
CAI-78	81	47	22	152	2.42	565
KAI-26	85	45	23	147	2.18	607
KAI-27	73	46	23	149	2.38	564
Control	72	36	21	120	2.03	484
Mean	76	42	22	138	2.19	555
SE±	0.5***	0.7***	0.3*	3.2**	0.032***	12.4***
LSD (5%)	1.6	2.6	1.0	11.7	0.116	39.0
CV%	1	3	2	3	2	4

Table 4. Gopalakrishnan et al. 2013

Treatment	Stover yield (g m^{-2})	Grain yield (g m^{-2})	Total dry matter (g m^{-2})
CAI-17	1452	788	2240
CAI-68	1493	787	2280
CAI-78	2133	872	3005
KAI-26	1302	981	2283
KAI-27	1344	813	2157
Control	1118	782	1899
Mean	1474	837	2311
SE±	62.6***	11.4***	65.2***
LSD (5%)	197.4	35.9	205.4
CV%	7	2	5

Table 5. Gopalakrishnan et al. 2013

Treatment	Root length (mm^{-2})			Root volume ($cm^3\ m^{-2}$)			Root dry weight (gm^{-2})		
	0−15 cm	15−30 cm	Mean	0−15 cm	15−30 cm	Mean	0−15 cm	15−30 cm	Mean
CAI-17	7106	950	4028	2108	189	1148	117.3	8.3	62.8
CAI-68	6474	1416	3945	2078	194	1136	136.0	9.7	72.9
CAI-78	7415	1341	4378	2501	194	1348	163.0	10.2	86.6
KAI-26	7180	1117	4148	2090	203	1147	117.0	8.2	62.6
KAI-27	8119	950	4535	2211	158	1184	144.2	7.3	75.8
Control	6355	425	3390	1692	72	882	106.2	3.6	54.9
SE±	126.9(29.3)***		125.2***	47.5(44.2)***		35.8***	3.34(2.97)***		2.59***
Mean	7108	1033		2113	168		130.6	7.9	
SE±	11.9***			18.0***			1.21***		
CV%	2			7			7		

Table 6. Gopalakrishnan et al. 2013

Treatment	Microbial biomass C (µg g^{-1} soil)	Microbial biomass N (µg g^{-1} soil)	Dehydrogenase activity (µg TPF g^{-1} soil 24 h^{-1})	Total N (ppm)	Available P (ppm)	Organic carbon (%)
CAI-17	3404	74.5	88	1766	99	1.26
CAI-68	2943	62.1	71	1866	98	1.30
CAI-78	3374	42.3	72	1544	96	1.35
KAI-26	3028	73.0	172	2595	115	1.47
KAI-27	2830	19.0	78	1855	90	1.50
Control	2300	17.7	62	1426	80	1.08
Mean	2980	48	90	1844	96	1.33
SE±	164.1**	6.66***	3.9***	33.5***	2.6***	0.060**
LSD (5%)	517.1	20.9	12.2	105.4	8.3	0.188
CV%	10	24	7	3	5	8