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HPLC detection of different isomers of catechins in tea leaves induced by single and dual application of *Bacillus megaterium* and *Serratia marcescens*

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Catechins are major flavor flavonoid components of tea and their quantitative changes with respect to different isomeric forms were analysed by HPLC. New isoforms and increase of isomers were observed in *Bacillus megaterium* (TRS 7) treated plants where as few were lost or there was suppression of few isomers by the treatment of *Serratia marcescens* (TRS 1). Similar trends were observed in TV26, TV18, TV25 and T17. But in 8 yr old plants of T-17/154, some new isomers were enhanced by the treatment of *S. marcescens.* Gallo catechin gallate (GCG), Gallocatechin (GC), Epigallo catechin gallate (EPC) and Epigallo catechin (EGC) with retention times of 13.13, 4.59, 10.95, and 5.92 mins respectively were detected in different varieties of tea induced by single as well as dual application of *B. megaterium* and *S. marcescens*. Major changes in the peaks were observed when inoculated by *B. megaterium,* all the peaks were of high intensity and few new peaks were observed with respect to control. However, no major loss of isomers were noted due to treatments indicating that flavor components were not lost.

Key words: Catechins, Gallo catechin gallate (GCG), Epigallo catechin gallate (EPC), Epigallo catechin (EGC), HPLC, tea, PGPR.

INTRODUCTION

Tea, derived from leaves of the plant *Camellia sinensis*, is the most widely consumed beverage in the world. It contains a wide variety of secondary metabolites, such as alkaloids, saponins, tannins, catechins, and polyphenols, generated through a condensation reaction of cinnamic acid with three malonyl-CoA groups. Catechins, the main component of polyphenols, are well known for their antioxidant properties, which have led to their evaluation in many diseases associated with free radicals, including cancer, cardiovascular diseases (Mukhtar and Ahmad 2000; Yang *et al.,* 2002).

Generally, the major catechins of tea leaves are (+)-Catechin (C), (")-Epicatechin (EC), (+)-Gallocatechin (GC), (")-Epigallocatechin (EGC), (")-Epicatechin gallate (ECg), (")-Epigallocatechin gallate (EGCg) and (+)-Gallocatechin gallate (GCg). Epigallocatechin-3-gallate (EGCG) is a catechin monomer found in tea leaves and accounts for 30% to 60% of the total phenolic compounds in tea leaves (Wang *et al.,* 2003). Plant growth promoting bacteria (PGPR) have the ability to promote growth in plants, which in many cases is associated with pathogen suppression in the soil. These PGPR secrete one or more metabolites in the soil which then elicit the observed response in the host. Whether it is growth promotion or disease suppression, the ultimate expression is in the host. Thus, these microorganisms or their products have the ability to elicit responses at molecular level which would include activation of a number of metabolic pathways in the host, the end product of which is finally expressed as increased growth of plant or reduced disease. PGPR may trigger flavonoid biosynthesis as part of an induced systemic response (ISR). Accumulation of higher

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levels of phenolics in plants resistant to various stresses. Higher accumulation of phenolics was observed in plants pre -treated with *Pseudomonas fluorescens* (PcPA23) and *Bacillus subtilis* (BsCBE4) challenged with *Pythium aphanidermatum* (Kavitha *et al.,* 2012).

The present study describes a sensitive and accurate high-performance liquid chromatography (HPLC) method to determine different isomers of catechins in tea varieties which are induced by the application of two potent PGPR. Accumulations of catechins, commonly known to be involved in the induced systemic resistance, have been enhanced by the mechanism of action of PGPR that secrete metabolites into the soil which in turn elicit responses in the host tea plant.

MATERIALS AND METHODS

Selection of tea varieties

For clonal propagation of tea plants, initially nodal cuttings of 15 varieties (TV- 23, TV- 26, TV-18, TV-29, TV-25, TV-30, TV-22, T-17, BSS-2, UP -9, UP -2, P-1258, K-1/1, AV-2 and S-449) were made and these were planted in sleeves. The plant varieties were originally collected from Tea Experimental Station, Tocklai (TV- 23, TV- 26, TV-18, TV-29, TV-25, TV-30, TV-22 and T-17), Darjeeling Tea Research Centre, Darjeeling (K1/1, P-1258, S 449 and AV-2) and United Planters Association of South India, Tamil Nadu (UP-9 and UP-2). Finally out of 15 varieties, four varieties (TV-18, TV-25, TV-26 and T-17) were selected for the present study. In the experimental plot of commercial tea garden, 8 yr old plants of T-17/1054 were also undertaken for analysis.

Source of bacterial cultures

Two bacterial isolates were isolated from the rhizosphere soil of tea bushes from Nagrakata Tea estate and Hansqua Tea Estate, respectively. They were also preliminarily identified on the basis of morphological, microscopic and biochemical characterization, and finally identity of these two strains were confirmed from the Plant Diagnostic and Identification Services, UK and also by 16S rDNA sequencing.

In vitro characterization of plant growth promoting activities of bacterial cultures IAA production

For detection and quantification of IAA, the selected bacterial cells were grown for 24 h to 48 h in high C/N ratio medium. Tryptophane (0.1 mM) was added in order to enhance acetic acid (IAA) production by the bacteria (Prinsen et al. 1993). Production of IAA in culture supernatant was assayed by Pillet-Chollet method as described by Dobbelaere *et al.,* (1999). For the reaction, 1 ml of reagent, consisting of 12 g $FeCl_3$ per litre in 7.9 M H_2SO_4 was added to 1 ml of sample supernatant, mixed well, and kept in the dark for 30 min at room temperature. Absorbance was measured at 530 nm.

Phosphate solubilisation

Primary phosphate solubilizing activities of *Bacterial cultures* were carried out by allowing the bacteria to grow in selective medium i.e., Pikovskaya' s agar (Himedia- M520; ingredients- yeast extract-0.50 g/l, dextrose10.00 g/l, calcium phosphate- 5.00 g/l, ammonium sulphate- 0.50 g/l, potassium chloride- 0.20 g/l, magnesium sulphate- 0.10 g/l, manganese sulphate- 0.0001 g/l, ferrous sulphate- 0.0001 g/l and agar- 15.00 g/l) for 7 to 10 days at 37°C (Pikovskaya 1948). The appearance of transparent halo zone around the bacterial colony indicated the phosphate solubilizing activity of the bacteria.

Siderophore production

The bacterial isolates were characterized for siderophore production following the method of Schwyn and Neiland (1987) using blue indicator dye, chrome azurol S (CAS). For preparing CAS agar, 60.5 mg CAS was dissolved in 50 ml water and mixed with 10 ml iron (III) solution (1 mM $FeCl_3 .6H_2O$ in 10 mM HCl) and volume made up to 1L. With constant stirring this solution was added to 72.9 mg hexadecyltrimethyl ammonium bromide (HDTMA), dissolved in 40 ml water. The resultant dark blue liquid was autoclaved. The dye solution was mixed into the medium along the glass wall with enough agitation to achieve mixing without the generation of foam, and poured into sterile petriplates (20 ml per plate). The plates were inoculated with the bacteria and incubated for 10-15 days till any change in the color of the medium was observed.

HCN production

Production of hydrocyanic acid was determined using the procedure described by Reddy *et al.,* (2008) with slight modification. The selected bacterial isolates were grown at room temperature (37^0C) on a rotary shaker in nutrient broth (NB) media. Filter paper (Whatman no.1) was cut into uniform strips of 10 cm long and 0.5 cm wide saturated with alkaline picrate solution and placed inside the conical flasks in a hanging position. After incubation at 37ºC for 48 hr, the sodium picrate present in the filter paper was reduced to reddish compound in proportion to the amount of hydrocyanic acid evolved. The color was eluted by placing the filter paper in a clean test tube containing 10 ml distilled water and the absorbance was measured at 625 nm.

Chitinase production

For detecting the chitinolytic behavior of the bacteria chitinase detection agar (CDA) plates were prepared by mixing 1.0% (w/v) colloidal chitin with 15 g of agar in a medium consisted of (Na_2 HPO_4 6.0 g, KH_2 PO_4 3.0 g, NaCl 0.5 g, NH_4Cl 1.0 g, yeast extract 0.05g and distilled water 1 L; pH 6.5). The CDA plate was spot inoculated with organism followed by incubation at 30°C for 7-10 days. Colonies showing zones of clearance against the creamy background were regarded as chitinase producing strains (Kamil *et al.,* 2007). The colloidal chitin was prepared by following the method described by Roberts and Selitrennikoff (1988). 5 g of chitin powder was slowly added to 60 ml of concentrated HCl and left at 4°C overnight with vigorous stirring. The mixture was added to 2 L of ice cold 95 % ethanol with rapid stirring and kept overnight at 25°C. The precipitation formed was collected by centrifugation at 7000 rpm for 20 min at 4°C and washed with sterile distilled water until the colloidal solution became neutral (pH 7). The prepared colloidal chitin solution (5 %) was stored at 4°C until further use.

Lipase production

Lipolytic activities of bacterial cultures were performed by allowing them to grow on spirit blue agar media. Lipase production by bacteria was assessed by the method of Marshall (1992).

Protease production

Protease activity was detected on 3% (wt/vol) powdered milk-agar plates according to Walsh *et al.,* (1995).

Application of bacteria

The prepared aqueous bacterial suspensions (final density of 2.8×10^9 cfu ml^{-1}) were applied as a soil drench as well as a foliar spray (bacterial suspensions along with a few drops of Tween-20), @ 100 ml/ plant to the rhizosphere of 2 yr old tea plants of TV-25, TV-18, TV-26 and T-17. Application was done at an interval of one month and three applications were done.

Similarly, bacterial cultures singly or jointly were applied as a soil drench, as well as foliar spray @ 100 ml/ plant to the rhizosphere of 8 yr old tea plants of T-17/1054. Application was done at an interval of two month and two applications were done.

Analysis of catechins Extracted from leaves

Extraction from tea leaf tissues was done following the method of Obanda and Owuor (1994) with slight modification. Leaf samples (10 g) were extracted with 100ml of acetone at 45°C in water bath for 30 min. Extracts were decanted and filtered through Whatman No.1 filter paper. Acetone extract was concentrated to dryness and finally the residue was dissolved in 20 ml distilled water. Aqueous solution was extracted with equal volume of chloroform for four times. The pH of the water layer was adjusted to 2 by 2 drops of 2 N HCl and finally extracted with methyl isobutyl ketone. The extract was concentrated to dryness and finally dissolved in 3 ml of 2 % acetic acid. The samples were finally filtered through milipore filter (Milipore 0.4μm HA filter paper).

HPLC analysis of catechins

Catechin analysis of the extract was carried out on HPLC (Shimadzu Advanced VP Binary Gradient) using C-18 hypersil column with linear gradient elution system as follows- mobile phase A 100 % acetonitrile; mobile phase B 2 % acetic acid in water. Elution: 88 % B for 6 min then linear gradient to 75 % B over 5 min. The elution was complete after 25 min. Flow rate was fixed as 1 ml min-1 with sensitivity of 0.5 aufs. Injection volume was 20 μl and monitored at 278 nm.

RESULTS AND DISCUSSION

Identification of bacterial culture

The BLAST query of 16S r DNA sequence of the isolates against GenBank database confirmed their identity. The sequences were deposited in NCBI, GenBank database under the accession Nos. JX 312687.1 and JN 020963.1 for *Bacillus megaterium* (TRS 7) and *S. marcescens* (TRS 1) respectively.

In vitro PGPR activities

In order to determine whether the bacteria possess plant growth promoting activities, initially several *in vitro* tests were conducted and results are presented below

Siderophore production

Siderophore production by bacterial strains was detected by growing the bacteria individually in chrome azurol S agar plate. The plates were observed for 10-15 days after inoculation with bacteria. The appearance of yellow halo region was observed around both *B. megaterium* and *S. marcescens* which indicated that both the bacterial isolates were able to chelate Fe^{3+} from chrome azurol S agar. The diameters of halo region were 1.8 cm and 2.0 cm for *B. megaterium* and *S. marcescens*respectively after 12 days of incubation (Table 1; Fig.1).

Phosphate solubilisation

Formation of clear zone around the colony grown in Pikovskaya's medium is an indication of phosphate solubilisation by rhizobacteria. In Pikovskaya's medium *B. megaterium* and *S. marcescens* produced clear zones of 1.7cm and 2.1cm diameter after 5-7 days of incubation, indicating that both the isolates could solubilise insoluble phosphate (Table 1; Fig.1).

Protease production

The bacteria were spot inoculated in Skim milk agar medium and incubated at 30ºC for 5-7 days. The appearance of clear region was observed around both *B. megaterium* and *S. marcescens* which indicated that both the bacterial isolates were able to produce protease. The diameters of clear zone were 2.8 cm and 3.9 cm for *B. megaterium* and *S. marcescens* respectively (Table 1; Fig.1).

IAA production

Both the bacterial strains were assessed for their ability to produce indole acetic acid by growing them in Nutrient Broth/ Luria Bertani Broth supplemented with tryptophane (0.1 mM). For quantification, HPLC analysis of IAA from *B. megaterium* and *S. marcescens* was done by injecting 10µl of the filtered extracts onto a (C18,5µm 25×0.46 cm) in a chromatograph equipped with a differential ultraviolet detector absorbing at 280 nm. Mobile phase was methanol: H_2O- 80:20(vol:vol), flow rate- 1.5ml/min. Retention times for peaks were compared to IAA standard (peak at retention time of 2.5 min for IAA standard) and quantified.*B. megaterium* recorded IAA production of 0.05 mg/ml. *S. marcescens* was found to produce 0.03 mg/ml (Table 1 and Fig.2).

Table 1: *In vitro* PGPR characteristics of *B. megaterium* and *S. marcescens*

Characteristics	*B. megaterium*	*S. marcescens*
Phosphate solubilisation	+	+
Siderophore production	+	+
Protease production	+	+
Chitinase production	-	+
HCN production	-	-
Volatile production	+	+
IAA production	+	+

+= activity present; - = activity absent

HCN production

To determine the ability of *B. megaterium* and *S. marcescens* to produce Hydrocyanic acid (HCN) the bacteria were grown in medium amended with glycine.

Results were observed after 4-7 days. Both *B. megaterium* and *S. marcescens* were found to be non-cyanogenic in nature (Table 1).

Chitinase production

The bacteria were spot inoculated in the 5 % colloidal chitin amended minimal medium and incubated at 30ºC for 7-10 days. It was observed that no extracellular chitinase was secreted by *B. megaterium* even when grown on chitin amended medium. But *S. marcescens* secreted extracellular chitinase indicating a clear zone around the colony of the bacterium on CDA plate (Table 1).

Table 2: Peak result of HPLCanalysis of catechin extracts from leaves of TV-25 following single and dual application of *Bacillus megaterium* and *Serratia marcecsens*

Peak result of HPLC analysis of catechin extracts from leaves of untreated control (cv. TV-25)

Peak no	Retn time (min)	Area(mVs)	Height(mV)	Area(%)	Height(%)
1	2.780	1048.4266	83.990	0.789	2.064
2	3.140	1065.4175	59.455	0.801	1.461
3	3.560	19206.9925	836.852	0.260	14.449
4	5.540	12201.7505	201.791	0.390	9.179
5	7.060	2606.2911	70.482	1.961	1.732
6	7.560	4774.7326	113.769	0.530	3.592
7	8.760	10906.6113	602.561	0.250	8.205
8	10.080	30207.5050	827.462	0.550	22.724
9	11.950	1818.4621	36.126	1.368	0.888
10	13.130	3409.1141	64.275	2.565	1.580
Peak result of HPLC analysis of catechin extracts from leaves of tea plants treated with *Bacillus megaterium*					
1	2.740	502.5549	42.927	0.294	0.699
2	3.110	965.0245	50.116	0.565	0.816
3	3.520	11939.7275	689.376	0.280	6.990
4	3.850	4169.9682	334.648	0.200	2.441
5	4.350	1553.2268	101.186	0.310	0.909
6	4.590	5191.5414	243.526	0.370	3.039
7	5.220	2875.4318	157.154	0.380	1.683
8	5.530	2579.3947	209.184	0.250	1.510
9	5.770	6234.7684	217.155	0.430	3.650
10	6.690	1613.8096	84.594	0.945	1.377
Peak result of HPLC analysis of catechin extracts from leaves of tea plants treated with *Serratia marcescens*					
1	2.590	203.6235	17.751	0.272	0.562
2	3.040	458.4001	17.911	0.612	0.567
3	3.530	9342.7823	513.677	0.230	12.474
4	4.450	458.2654	34.719	0.612	1.099
5	4.690	1584.3929	94.744	2.115	3.000
6	5.160	520.9088	34.945	0.695	1.107
7	5.470	894.5168	50.167	1.194	1.589
8	5.800	1326.3412	78.603	1.771	2.489
9	6.160	1567.2746	56.951	2.093	1.803
10	6.900	505.0251	23.650	0.674	0.749
Peak result of HPLC analysis of catechin extracts from leaves of tea plants treated with *Bacillus megaterium* and *Serratia marcescens*.					
1	2.690	430.4852	35.918	0.599	1.586
2	3.070	400.8424	22.389	0.557	0.989
3	3.570	9280.9945	389.386	0.280	12.904
4	4.680	1711.7022	78.215	2.380	3.453
5	5.750	4154.4133	83.398	5.776	3.682
6	6.880	366.1677	20.857	0.509	0.921
7	7.370	968.0418	33.623	1.346	1.485
8	8.100	2373.0443	54.892	3.299	2.424
9	9.200	4802.5770	257.904	0.250	6.677
10	10.950	14564.3647	394.621	0.550	20.249

Lipase production

B. megaterium and *S. marcescens* showed negative responses for lipolytic activities (Table 1).

Changes in catechin profiles in tea leaves induced by bacteria

Since plant growth promotion could also be due to induction of biochemical responses within the host, experiments were conducted to assess the effect

Table 3: Peak result of HPLC analysis of catechin extracts from leaves of TV-18 following single and dual application of *Bacillus megaterium* and *Serratia marcecsens*

Peak no	Retn time (min)	Area(mVs)	Height(mV)	Area(%)	Height(%)
Peak result of HPLC analysis of catechin extracts from leaves of untreated control					
1	2.640	1058.5157	64.966	0.969	1.433
2	3.070	758.1258	43.975	0.694	0.970
3	3.470	15313.8499	819.853	0.230	14.018
4	4.340	682.8369	49.535	0.625	1.093
5	4.700	2584.6250	117.747	0.380	2.366
6	5.270	2119.1078	99.097	1.940	2.186
7	5.600	1979.3081	139.076	0.300	1.812
8	5.910	2429.1389	98.098	2.224	2.164
9	6.540	683.1326	38.493	0.625	0.849
10	6.910	717.3183	44.817	0.657	0.989
Peak result of HPLC analysis of catechin extracts from leaves of tea plants treated with *Bacillus megaterium*					
1	3.520	10844.4371	618.435	0.210	15.874
2	4.700	2041.8730	98.327	2.989	3.049
3	5.260	1366.1923	68.431	2.000	2.122
4	5.580	3038.0797	117.364	0.280	4.447
5	8.890	9096.5958	558.570	0.240	13.316
6	10.600	11078.1562	484.786	0.330	16.216
7	14.750	2256.5553	92.106	3.303	2.856
8	15.300	977.1638	58.489	1.430	1.814
9	15.720	2328.7234	123.263	0.290	3.409
10	16.250	6487.2648	262.104	0.370	9.496
Peak result of HPLC analysis of catechin extracts from leaves of tea plants treated with *Serratia marcescens*					
1	3.540	9406.6806	533.144	0.210	14.103
2	4.710	2010.0994	92.416	3.014	2.990
3	5.800	1546.5727	100.826	0.270	2.319
4	6.180	1482.5404	53.170	2.223	1.720
5	9.210	7591.3571	441.171	0.240	11.381
6	11.310	11653.4481	476.128	0.360	17.472
7	15.000	2039.0612	75.283	3.057	2.435
8	15.530	1185.5679	61.689	1.777	1.996
9	15.930	1927.9808	98.347	2.891	3.181
10	16.520	9103.1640	353.298	0.210	13.648
Peak result of HPLC analysis of catechin extracts from leaves of tea plants treated with *Bacillus megaterium* and *Serratia marcescens*.					
1	3.510	3024.3928	226.453	0.220	3.843
2	3.810	2429.1077	138.699	0.260	3.087
3	4.650	5196.3155	398.848	0.190	6.603
4	5.390	1624.5667	70.453	2.064	1.952
5	5.710	2365.2179	150.305	0.270	3.006
6	9.190	7656.8056	449.990	0.240	9.730
7	11.030	13131.8767	433.201	0.440	16.688
8	14.980	2333.4112	90.903	2.965	2.519
9	15.450	2274.2960	98.060	2.890	2.717
10	15.890	2525.8460	139.816	0.380	3.210

of single as well as combined application of *B. megaterium* and *S. marcescens* on flavonoid flavour components of tea leaves. Catechins which are the flavonoid flavour component of tea leaves are extremely important, derived from leaves of plants whose rhizosphere was soil drenched with bacteria and changes in these were also analyzed by HPLC. Analysis revealed that a few isomers were enhanced by the treatments, a few new ones developed and few were lost. New isoforms and increase of isomers were observed in *B. megaterium* treated plants where as few were lost or there was suppression of few isomers by the treatment of *S. marcescens*. Similar trends were observed in TV26, TV18, TV25 and T17.

In TV-25 variety, in control one isomer- gallo catechin gallate (GCG) with retention time of 13.13 min, whereas, in *B.megaterium* treatment, two isomers- gallocatechin (GC) and gallo catechin gallate (GCG) with retention times of 4.59 and 13.36 min were detected. Similarly, in *S. marcescens* and *B.megaterium+S.marcescens* treated TV-25 variety, gallo catechin gallate (GCG)

Table 4: Peak result of HPLC analysis of catechin extracts from leaves of T-17 following single and dual application of *Bacillus megaterium* and *Serratia marcecsens*

Peak no	Retn time (min)	Area(mVs)	Height(mV)	Area(%)	Height(%)
Peak result of HPLC analysis of catechin extracts from leaves of untreated control					
1	2.730	923.8596	73.150	0.895	1.556
2	3.050	555.2647	32.947	0.538	0.701
3	3.490	3825.9065	312.279	3.707	6.643
4	3.840	3473.6993	245.323	0.200	3.365
5	4.350	916.2130	73.319	0.888	1.560
6	4.590	4279.0588	273.969	0.240	4.146
7	5.200	2434.4760	114.271	0.410	2.359
8	5.550	5681.1259	227.982	0.390	5.504
9	6.480	799.3863	43.253	0.774	0.920
10	6.780	772.0844	48.934	0.748	1.041
Peak result of HPLC analysis of catechin extracts from leaves of tea plants treated with *Bacillus megaterium*					
1	2.710	1138.6316	85.844	0.767	1.340
2	3.030	907.8586	52.192	0.612	0.815
3	3.480	4739.4171	360.210	0.200	3.194
4	3.800	4057.6172	257.431	0.230	2.734
5	4.340	1661.8404	113.188	0.300	1.120
6	4.570	11821.6223	748.179	0.230	7.966
7	5.230	2902.8866	149.618	0.390	1.956
8	5.540	8785.3124	344.754	0.430	5.920
9	6.410	1075.8097	59.820	0.725	0.934
10	6.780	1232.7278	80.906	0.831	1.263
Peak result of HPLC analysis of catechin extracts from leaves of tea plants treated with *Serratia marcescens*					
1	3.700	658.2046	47.614	1.442	2.286
2	4.030	387.9846	22.828	0.850	1.096
3	4.520	2678.2978	202.315	0.210	5.866
4	4.830	2740.2886	176.704	0.230	6.001
5	5.400	596.3365	48.530	1.306	2.330
6	5.660	2768.5244	185.246	0.220	6.063
7	6.390	1708.4239	66.726	3.742	3.204
8	6.720	2263.0383	137.578	0.280	4.956
9	7.130	1290.6704	53.139	2.827	2.552
10	8.280	745.6566	21.027	1.633	1.010
Peak result of HPLC analysis of catechin extracts from leaves of tea plants treated with *Bacillus megaterium* and *Serratia marcescens*.					
1	2.670	611.9642	41.567	0.752	1.140
2	3.020	462.8038	27.251	0.569	0.747
3	3.550	4761.5013	174.024	5.849	4.772
4	4.180	492.1371	40.596	0.605	1.113
5	4.470	655.3570	57.515	0.805	1.577
6	4.730	6045.2400	413.254	7.427	11.332
7	5.250	635.0962	44.612	0.780	1.223
8	5.550	1322.1169	73.307	1.624	2.010
9	5.870	2955.4777	172.419	0.280	3.631
10	6.310	1980.1466	78.325	2.433	2.148

and epigallo catechin gallate (EPC) with retention times of 13.36 and 10.95 min were detected. In TV-18 variety, in addition to gallo catechin gallate (GCG) with retention time of 13.36 min, another isomer-epigallo catachin (EGC)- 5.922 was detected in control, *B. megaterium* and *B.megaterium+S.marcescens* treated plants (Tables 2 and 3 and Figs. 3 and 4).

Similarly, in T-17 and TV-26 varieties, gallo catechin (GC) - (4.59-control; 4.59- *B. megaterium* treated), gallo catechin gallate(GCG)-(13.36- in control; *B. megaterium* and *B.megaterium+S.marcescens* treated) and epigallo catechin (EGC) with retn. time- 5.92 (in *B.meg-aterium+S.marcescens* treated plants) were predicted as isomers of catechin (Tables 4&5 and Figs. 5&6). However, no major loss of isomers were noted due to treatments indicating that flavor components were not lost. Catechins EGCG, EGC, ECG, EC and gallocatechin-3-gallate (GCG) were evidenced to play an important role in green tea's inhibition of bacterial growth, involving damage in bacterial cell membranes (Reygaert 2018).

Table 5: Peak result of HPLC analysis of catechin extracts from leaves of **TV-26** following single and dual application of *Bacillus megaterium* and *Serratia marcecsens*

Peak no	Retn time (min)	Area(mVs)	Height(mV)	Area(%)	Height(%)
Peak result of HPLC analysis of catechin extracts from leaves of untreated control					
1	2.740	590.1520	40.825	0.365	1.106
2	3.670	12722.5907	398.654	0.500	7.874
3	4.700	5811.0867	233.824	0.300	3.597
4	5.740	12776.1564	228.421	0.920	7.907
5	7.120	3561.0949	80.059	2.204	2.169
6	8.040	4391.8700	95.650	2.718	2.591
7	9.040	12202.0500	457.589	0.380	7.552
8	11.050	24748.7733	510.511	0.720	15.317
9	12.040	2267.0321	85.013	1.403	2.303
10	12.530	1489.7794	53.348	0.922	1.445
Peak result of HPLC analysis of catechin extracts from leaves of tea plants treated with *Bacillus megaterium*					
1	2.770	1390.6953	112.542	0.506	1.853
2	3.180	2298.4376	143.713	0.837	2.366
3	3.500	20019.4371	617.316	7.287	10.163
4	4.740	12517.9887	515.993	4.556	8.495
5	5.750	22863.1957	503.242	8.322	8.285
6	7.430	6008.6941	178.511	2.187	2.939
7	8.120	12534.9687	336.524	4.562	5.541
8	9.110	23621.9931	630.417	8.598	10.379
9	10.490	47348.2430	628.679	17.233	10.351
10	12.240	3360.0023	122.606	1.223	2.019
Peak result of HPLC analysis of catechin extracts from leaves of tea plants treated with *Serratia marcescens*					
1	2.680	646.6168	50.319	0.648	1.311
2	3.090	607.9323	36.357	0.609	0.947
3	3.470	12878.1841	610.034	0.250	12.907
4	4.390	718.7049	49.757	0.720	1.297
5	4.640	2571.7516	145.085	0.250	2.577
6	5.390	1850.6808	73.181	1.855	1.907
7	5.710	2008.9874	120.983	0.360	2.013
8	6.040	1845.5117	68.265	1.850	1.779
9	6.780	544.2133	29.867	0.545	0.778
10	7.280	1354.6690	47.139	1.358	1.228
Peak result of H PLC analysis of catechin extracts from leaves of tea plants treated with *Bacillus megaterium* and *Serratia marcescens*.					
1	2.700	417.5193	28.562	0.508	1.078
2	3.350	593.0862	34.997	0.721	1.320
3	3.610	8441.8778	373.113	0.280	10.266
4	4.780	2142.1280	125.737	0.220	2.605
5	5.230	977.0494	37.955	1.188	1.432
6	6.080	2619.4997	86.591	3.186	3.267
7	6.570	1740.8535	53.279	2.117	2.010
8	7.440	377.4445	22.820	0.459	0.861
9	7.790	1130.3766	39.000	1.375	1.471
10	8.770	2272.4421	48.318	2.764	1.823

Coordinated expression of flavonoid biosynthetic genes with the accumulation of catechins and

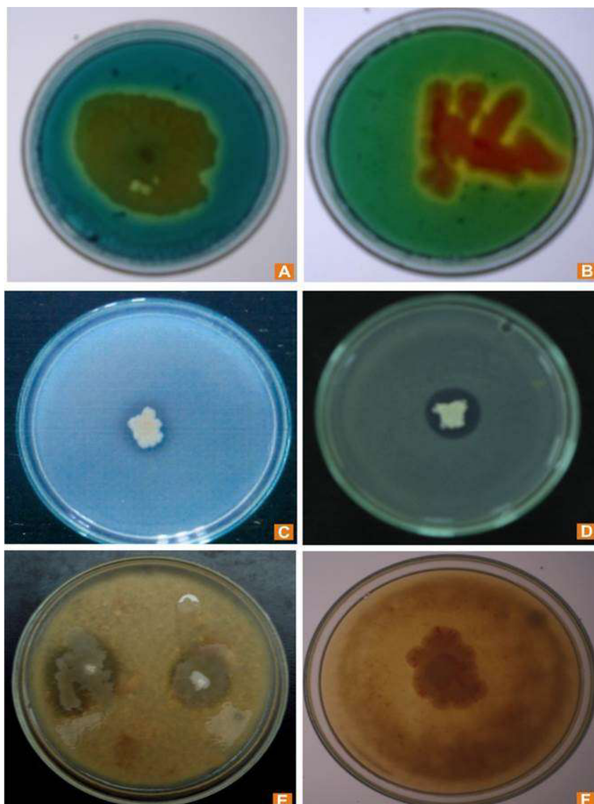

Fig. 1 : *In vitro* PGPR activities of *B. megaterium* (A,C,E) and *S. marcescens* (B,D,F). A&B-siderophore production, C&D-Phosphate solubilization in PKV medium, E&F-Protease production in skim milk agar medium.

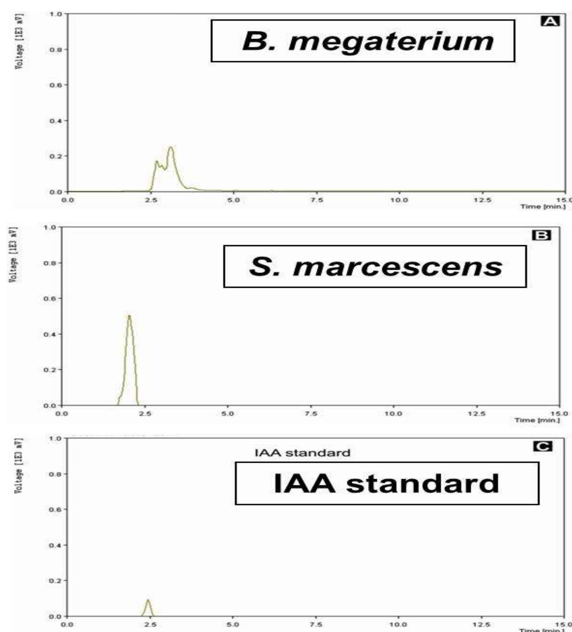

Fig. 2 : HPLC profile of IAA from *B. megaterium* (A) and *S. marcescens* (B) along with IAA standard (C).

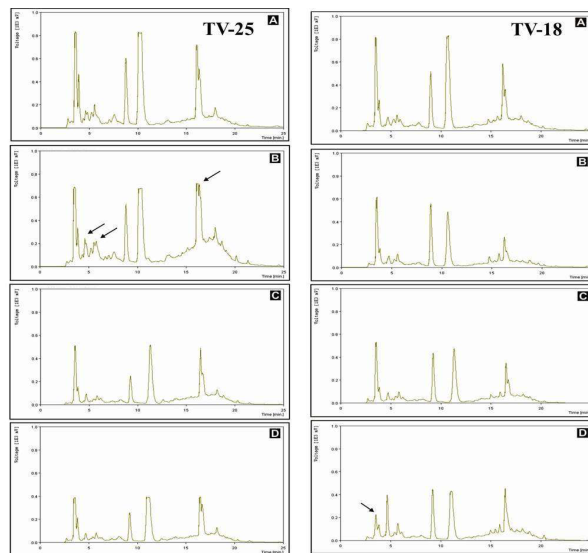

Fig. 3 Fig. 4

Fig. 3 : HPLC profiles of catechins of tea leaves treated with *B. megaterium* (B), *S. marcescens* (C) and *B. megaterium*+ *S. marcescens* (D) in comparison to untreated leaves (A) of TV 25. (Arrows indicate new isoforms and increase of isomers in treated plants) & **Fig. 4:** HPLC profiles of catechins of tea leaves treated with *B. megaterium* (B), *S. marcescens* (C) and *B. megaterium*+ *S. marcescens* (D) in comparison to untreated leaves (A) of TV 18. (Arrows indicate new isoforms and increase of isomers in treated plants).

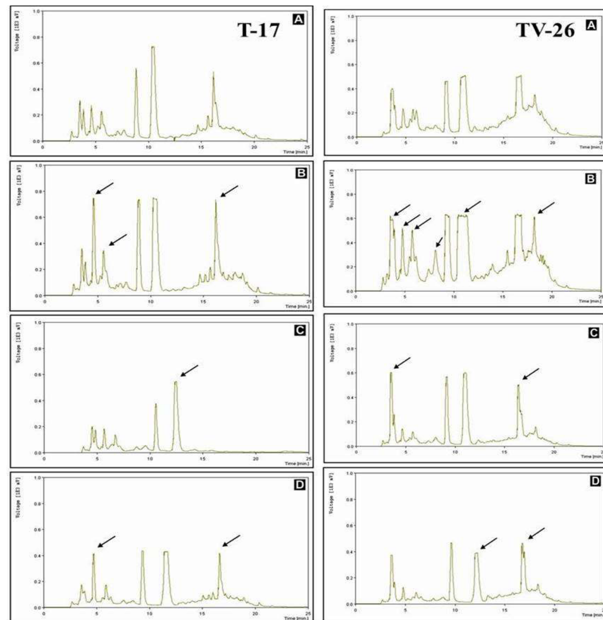

Fig. 5 Fig. 6

Fig. 5 : HPLC profiles of catechins of tea leaves treated with *B. megaterium* (B), *S. marcescens* (C) and *B. megaterium*+ *S. marcescens* (D) in comparison to untreated leaves (A) of T 17. (Arrows indicate new isoforms and increase of isomers in treated plants) & **Fig. 6:** HPLC profiles of catechins of tea leaves treated with *B. megaterium* (B), *S. marcescens* (C) and *B. megaterium*+ *S. marcescens* (D) in comparison to untreated leaves (A) of TV 26. (Arrows indicate new isoforms and increase of isomers in treated plants).

flavonols were observed in developing fruits of blackberry by the application of PGPR-*Pseudomonas fluorescens* (Daniel *et al.,* 2015).

CONCLUSION

The overall results depict the role of PGPR in induction of different isoforms of catechin in tea. *Bacillus megaterium* (TRS 7) showed comparatively better response in inducing major changes in the peaks with higher intensity.

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