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Studies on the effect of plant growth promoting rhizobacteria (PGPR) on growth, physiological parameters, yield and fruit quality of strawberry cv. chandler

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Abstract
An experiment was conducted to investigate the effective use of plant growth promoting rhizobacteria (*Bacillus licheniformis* CKA 1, *Bacillus subtilis* CB 8 A, *Bacillus sp.* RG1, *Bacillus sp.* S_1 and *Bacillus sp* S_2) on growth, yield and fruit quality of strawberry cv. Chandler at Model Farm of Directorate of Research, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India during 2012-2013. It was observed that the root+ foliar application of plant growth promoting rhizobacteria gave the best results with respect to growth, yield and fruit quality as compared to other application methods. The maximum plant height and spread were recorded in T_3 whereas the maximum leaf area, chlorophyll content, rate of photosynthesis, stomatal conductance and transpiration rate were observed in T_{15}. The number of crowns were maximum in T_9 followed by T_{15}, while the number of runners were significantly more in T_{12} showing significant improvement over control. The highest number of fruits and yield per plant were highest in T_{15}, respectively. The maximum fruit weight and length were recorded from T_{15} while fruit diameter was maximum T_3. The maximum fruit TSS in T_{13}, TSS: acid ratio in T_4, acidity in control over other treatments. Total sugars in T_{10}, Reducing Sugars were highest in T_7 the as compared to other treatments. The ascorbic acid content was highest in T_{16} while anthocyanin content was maximum in T_1 as compared to other treatments.

Keywords: strawberry, plant growth promoting rhizobacteria, growth, yield, physiological parameters and fruit quality

Introduction
The strawberry *(Fragaria × ananassa)* is one of the most popular and choicest berry fruits in the world owing to its flavour, deliciousness, softness and rich source of mineral and nutrients. The crop is in great demand for fresh fruits as well as in the processing industries because the low-growing strawberry plants are reliable, nutrient rich and quick to produce.
Haryana is leading state in strawberry cultivation followed by Mizoram, Meghalaya, Maharashtra, Himachal Pradesh, Uttrakhand and Jammu & Kashmir in India (Anonymous, 2017a) [1]. In Himachal Pradesh, the crop occupies an area of 54 hectare with annual production of 84 MT (Anonymous, 2017b) [2]. Although, strawberry cultivation is becoming popular in Himachal Pradesh, but due to lack of proper nutrient management, farmers are usually getting poor quality fruits and yield. In order to get high yield and quality fruits, improved management practices like use of plant growth promoting rhizobacteria have been becoming a resurgence of interest in sustainable and organic cultural practices as they have been found to increase plant growth, yield and quality by the means of producing plant growth regulators (auxin, gibberellins, cytokinins etc.), mineralizing complex nutrients, fixing atmospheric nitrogen, facilitating the uptake of nutrients and preventing deleterious effects on soil in many fruit crops (Singh *et al.*, 2017a [3], Esitken *et al.*, 2005 [4],Pandit *et al.*, 2013 [5], Tripathi*et. al.*, 2014 [6], Singh and Sharma 2017a [7], Ipek *et al.*, 2014 [8]. Moreover, they are cost effective and renewable. In view of the above, the present investigation was undertaken to study the effect of plant growth promoting rhizobacteria on growth, yield and fruit quality of strawberry cv. Chandler.

Material and Methods
The present investigation was conducted at Model Farm of Directorate of Research, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan, (HP), India during 2012-13. The experiment was laid out as the randomized block design (RBD) with sixteen treatments, each with three replications consisting 48 beds (2x2 m). The planting of strawberry cultivar

'Chandler' runners was done during October, 2012 at a spacing of 50 x 25 cm. The five plant growth promoting rhizobacteria (PGPR) {S_1: *Bacillus licheniformis* CKA 1 (10^9cfu/ ml), S_2: *Bacillus subtilis* CB 8 A (10^9cfu/ ml), S_3: *Bacillus sp.* RG1 (10^9cfu/ ml), S_4: *Bacillus sp.* S_1 (10^9cfu/ ml) and S_5: *Bacillus sp*S_2 (10^9cfu/ ml)} were applied as root dip, foliar application and root dip+ foliar application methods along with control (sterile water application) viz., T_1: S_1 + Root dip method, T_2: S_1+ Foliar application, T_3: S_1 + Root dip method + Foliar application, T_4: S_2+ Root dip method, T_5: S_2+ Foliar application, T_6: S_2 + Root dip method + Foliar application, T_7: S_3 + Root dip method, T_8: S_3+ Foliar application, T_9: S_3 + Root dip method + Foliar application, T_{10}: S_4+ Root dip method, T_{11}: S_4+ Foliar application, T_{12}: S_4 + Root dip method + Foliar application, T_{13}: S_5 + Root dip method, T_{14}: S_5+ Foliar application, T_{15}: S_5 + Root dip method + Foliar application and T_{16}: Control. The plant growth promoting rhizobacteria were applied as root dip method at the time of planting and foliar spray was done 20 days before expected flowering.

The treated plants were evaluated to see the effect of plant growth promoting rhizobacteria on growth and yield i.e. height (cm), plant spread (cm), number of crowns, number of runners, number of fruits and yield per plant (g) as per standard practices. The leaf area was measured with the help of LICOR- Model 3100. The physiological parameters such as chlorophyll content (mg/ g) was determined by the method suggested by Hiscox and Israelstam (1979) [9], while the photosynthesis, stomatal conductance, resistance and transpiration were recorded during fruit development stage with the help of LICOR-6200 portable photosynthesis meter and expressed in µ mol/m^2/s, m mol/s, S cm^{-1} and m mol/m^2/s. The effects on fruit quality were evaluated by determining, fruit weight (g), fruit size (mm), total soluble solids (TSS), titrable acidity, TSS: acidity ratio, total sugars, reducing sugars, non-reducing sugars, ascorbic acid and anthocyanin content as per A.O.A.C method (1980) [10], Rangana (2010) [11], respectively. Statistical analysis of the data was carried out by the method of analysis of variance as outlined by Gomez and Gomez (1984) [12].

Result and Discussion

Plant growth and yield

Application of plant growth promoting rhizobacteria significantly increased the vegetative growth in strawberry plants when applied through root dip + foliar application method. It is evident from the Table 1 that the maximum plant height (29.50 cm) and plant spread (31.03 cm) were recorded in T_3, whereas the leaf area (130.67cm^2) was maximum in T_{15} followed by T_3 (129.90 cm^2). An increase in number of crowns (4.62) with the application of plant growth promoting isolates was observed in T_9 while, the number of runners were highest in T_{12} as compared to other treatments. This significant increase may be due to the possibility of plant growth promoting rhizobacteria to produce plant growth hormones, better translocation of water and nutrient, efficient uptake and utilization of water and nutrients. The results are in agreement with the previous report of Kandan (2000)[13] who observed increased leaf area and shoot length, in tomato (*Lycopersiconesculentum* Mill.) plants that had been treated with a consortium of three different *P. fluorescens* strains, CHAO, CoT1 and CPO1. They reported increased leaf areas and plant biomass with the application of plant growth promoting rhizobacteria. The increase in leaf area and plant biomass could be attributed to increased mineral nutrition and the possibility of plant growth promoting rhizobacteria to produce plant growth hormones, increased photosynthesis, better root development, better translocation of water and nutrients, uptake and efficient utilization of nutrients (Singh and Sharma (2017b) [14], Singh *et al.*, (2017b) [15]. The findings of present research are in line with the work of Pirlak and Kose (2009) [16], Singh *et al.*, (2017c) [17], Yadav *et al.*, (2010) [18], Tripathi and Gupta (2012) [19], Karlidag *et al.* (2013) [20].

The maximum number of fruits ((18.07) and yield per plant (253.64 g) were obtained with the application of *Bacillus sp*S_2 when applied through root dip + foliar application methods at T_{15} and minimum (13.20 and 161.43 g, respectively) in T_{16}. It could be due to the production of auxin and cytokinin by plant growth promoting isolates may have affected cell division, floral initiation and development directly or indirectly resulting in higher number of fruits and yield. The results are in conformity with the findings of Tarenghi and Martin (1995) [21], Esitken*et al.* (2010) [22] and Ipek *et al.* (2014) [8].

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Leaf physiological parameters

The plant growth promoting rhizobacteria had shown significantly positive effects on leaf physiological parameters in treated plant as compared to untreated plants. The increased chlorophyll content (3.01 mg/g), rate of photosynthesis (9.77 µ mol/m^2/s), stomatal conductance (0.606 m mol/s), transpiration rate (38.80 m mol/m^2/s) and decreased stomatal resistance (0.597 S cm^{-1}) were recorded from T_{15} while the lowest stomatal resistance (1.242 S cm^{-1}) was recorded in untreated plants (T_{16}). It appears that the increase in chlorophyll content might be the result of increased leaf area and balanced nutritional environment in soil by keeping iron physiologically active as reported by EI Morshedy (1997) [23]. The increase in photosynthesis, stomatal conductance, transpiration and decreased stomatal resistance can be attributed to increased leaf area, chlorophyll content and strong source- sink relationship. The increase in photosynthesis, chlorophyll florescence, stomatal conductance and transpiration rate, and decreased stomatal resistance can be attributed to increased leaf area, chlorophyll content and strong source- sink relationship. Sharma and Sharma (2008) [24] also recorded variable effects of rootstocks on the scion leaf transpiration rate and stomatal resistance. Plant growth hormones produced by rhizobacterial strains have also been found to increase the N-use efficiency and activities of nitrate reductase (NR) and carbonic anhydrase (CA) of plants. The increased N utilization by plants also help in increased photosynthesis. The increase in photosynthesis, stomatal conductance, transpiration rate and decreased stomatal resistance may be result of increased chlorophyll content, stomatal opening and CO_2 assimilation (Misratia*et al.*, 2013) [25]. The findings are in agreement with the work of De Veau*et al.*, (1990) [26], Osman and El-Rhman (2010) [27], Singh *et al.*, (2012) [28], Stefan *et al.*, (2013) [29].

Fruit quality

The given data (Table 3) reveals that the fruit quality was significantly influenced by application of plant growth promoting rhizobacterial isolates. The maximum fruit weight (14.62 g) and length (39.14 mm) were recorded in T_{15} while fruit diameter (28.33 mm) was maximum in T_3. The TSS (10.91 ^{0}Brix) was highest in T_{13} and the TSS: acidity ratio (8.87) in T_4, whereas the acidity (1.91 %) and ascorbic acid (55.33 mg/ 100g) were maximum in T_{16}. The maximum anthocyanin content (40.83 mg/ 100 ml) was recorded in T_1 as

compared to other treatments. The highest total sugars (6.70 %) were recorded in T_{10} and reducing Sugars (5.20 %) in T_7 whereas the non-reducing sugars (2.36 %) were obtained from T_2. This increase can presumely be due to increased leaf area, more photosynthesis, enhanced carbohydrates accumulation and efficient partitioning of photosynthates towards the sink stimulated by plant growth promoting rhizobacteria. Contrary, the decrease in acidity and ascorbic may be due to increase in accumulation of more assimilate or total soluble solids in fruits. The increase in total soluble solids, sugars and anthocyanin can presumely be due to the more availability of assimilate as a result of increased photosynthesis and nutrient availability to the fruit plants as results of application of plant growth promoting rhizobacteria. Contrary, the decrease in acidity and ascorbic may be due to increase in accumulation of more assimilate or total soluble solids. Similar results for decrease in acidity and ascorbic acid with the application of plant growth promoting rhizobacteria also observed by the Esitken *et al.*, (2010) [22] where control treatment (0.70 % and 71.58 mg/100 ml, respectively) provided the highest per cent acidity and vitamin C content. The results are in conformity with the findings of Pirlak and Kose (2009) [16] who reported that the root application of plant growth promoting rhizobacteria (PGPR) strains significantly increased total soluble solids (8.15 %), total sugar (5.90 %) and reducing sugar (4.69 %) but decreased titratable acidity (0.82 %) in strawberry cultivar Selva. Umar *et al*. (2009) [30] recorded increased anthocyanin content (0.191 OD) with the application of Azotobacter in combination with FYM + urea in strawberry.

Table 1: Effect of plant growth promoting rhizobacteria on growth and yield of strawberry cultivar Chandler

Treatment	Plant height (cm)	Plant spread (cm)	Leaf area (cm^2)	Number of crowns/ plant	Number of runners/ plant	Number of fruits/ plant	Yield per plant (g)
T_1	24.03	26.78	125.07	3.78	35.58	14.82	192.15
T_2	25.52	25.97	115.52	3.50	31.75	16.10	229.50
T_3	29.50	31.03	129.90	4.18	40.00	16.73	234.21
T_4	23.33	27.90	109.83	3.73	30.92	14.53	186.73
T_5	25.56	26.93	108.97	3.44	28.09	16.40	214.72
T_6	25.96	29.92	110.27	4.00	32.54	16.60	223.48
T_7	22.87	25.06	110.12	4.38	36.43	15.27	185.07
T_8	23.42	24.34	107.85	4.08	28.17	16.93	211.94
T_9	25.70	28.18	125.05	4.62	38.79	17.27	214.09
T_{10}	23.17	26.34	111.18	3.87	38.09	14.53	182.31
T_{11}	26.15	25.95	107.78	3.82	28.83	15.47	208.94
T_{12}	26.63	27.40	116.55	4.08	48.43	15.80	210.38
T_{13}	22.92	24.13	121.89	4.07	31.58	16.53	222.03
T_{14}	23.85	23.37	120.48	3.85	28.67	17.33	251.89
T_{15}	27.48	28.45	130.67	4.44	36.40	18.07	253.64
T_{16}	22.20	23.05	104.70	3.33	22.25	13.20	161.43
C.D. $_{0.05}$	2.19	1.92	2.96	0.51	2.84	2.08	1.93

Table 2: Effect of plant growth promoting rhizobacteria on physiological parameters of strawberry cultivar Chandler

Treatment	Chlorophyll Content (mg/ g)	Photosynthesis (µ mol/m^2/s)	Stomatal Conductance (m mol/s)	Stomatal resistance (S cm^{-1})	Transpiration rate (m mol/ m^2/s)
T_1	2.70	5.68	0.422	1.162	24.02
T_2	2.76	7.93	0.461	1.110	24.39
T_3	2.93	8.22	0.510	0.997	26.79
T_4	2.71	6.02	0.421	1.103	25.41
T_5	2.78	6.23	0.423	0.890	27.83
T_6	2.90	6.83	0.458	0.840	29.67
T_7	2.53	6.55	0.425	1.135	24.07
T_8	2.61	7.16	0.444	0.955	26.98
T_9	2.95	9.04	0.570	0.739	30.10
T_{10}	2.59	6.71	0.438	0.822	29.78
T_{11}	2.67	6.75	0.441	0.733	33.82
T_{12}	2.72	6.94	0.449	0.636	34.86
T_{13}	2.69	6.56	0.455	0.838	28.11
T_{14}	2.71	7.68	0.503	0.682	34.42
T_{15}	3.01	9.77	0.606	0.597	38.80
T_{16}	2.51	5.43	0.372	1.242	22.76
C.D. $_{0.05}$	0.10	0.57	0.041	0.082	0.57

TABLE 3: Effect of plant growth promoting rhizobacteria on physico-chemical properties of strawberry cultivar Chandler

Treatment	Fruit weight (g)	Fruit Length (mm)	Fruit Diameter (mm)	Anthocyanin content (mg/ 100 ml)	TSS (^{0}Brix)	Titrable acidity (%)	TSS: Acidity Ratio	Ascorbic acid (mg/100 g fruit weight)	Total sugars (%)	Reducing sugars (%)	Non-reducing sugar (%)
T1	12.66	37.27	27.47	40.83	10.85	1.41	7.72	31.30	6.44	4.08	2.24
T2	13.76	37.45	27.83	30.50	9.60	1.56	6.20	38.33	5.78	3.30	2.36
T3	14.43	38.04	28.33	32.12	9.64	1.47	6.63	35.34	5.97	3.65	2.21
T4	12.82	36.98	26.96	38.83	10.73	1.21	8.87	37.50	5.85	4.77	1.03

T5	12.94	37.44	27.00	29.06	9.66	1.70	5.69	52.50	5.46	3.95	1.44
T6	13.63	37.70	27.73	30.31	9.86	1.52	6.61	41.00	5.60	4.53	1.02
T7	12.16	36.17	26.64	38.17	10.64	1.25	8.56	39.17	6.54	5.20	1.27
T8	12.41	36.52	26.94	30.49	9.43	1.55	6.23	47.08	5.06	3.76	1.23
T9	12.63	38.15	27.45	34.90	9.56	1.34	7.56	43.83	6.18	4.37	1.72
T10	12.56	34.09	25.48	38.58	9.99	1.36	7.43	41.17	6.70	4.64	1.95
T11	13.28	37.12	26.97	32.92	9.63	1.47	6.57	52.08	6.02	3.72	2.19
T12	13.60	37.52	27.16	37.64	9.80	1.41	7.01	43.78	6.24	4.04	2.09
T13	13.46	36.32	27.74	39.17	10.91	1.42	7.76	45.42	6.14	4.89	1.18
T14	13.98	38.53	28.05	29.61	9.84	1.70	5.94	49.58	5.11	3.79	1.26
T15	14.62	39.14	28.12	36.14	10.36	1.66	6.25	48.57	5.92	4.07	1.76
T16	11.17	32.62	25.16	27.85	9.01	1.91	4.80	55.33	4.68	3.25	1.36
C.D. $_{0.05}$	0.90	1.29	0.96	1.43	0.67	0.20	1.00	1.06	0.67	0.69	0.90

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