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Research Article

IMPACT OF PGPR AND GA_3 ON GROWTH, YIELD AND LEAF NUTRIENT STATUS OF STRAWBERRY

KUMARI SEEMA[1*] AND MEHTA KULDEEP[2]
[1]Department of Agriculture, Baba Farid College, BFGI, Deon, Bathinda, Punjab, 151001, India
[2]Department of Fruit Science, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, 173230, India
*Corresponding Author: Email - samilodott@gmail.com

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Abstract: A field trial was conducted to monitor the impact of five isolates of *Bacillus* sp. *viz*. *Bacillus licheniformis* CKA1, *Bacillus subtilis* CB 8 A, *Bacillus* sp. RG1, *Bacillus* sp. S_1 and *Bacillus* sp. S_2 @ 109 CFU in combination with GA_3 (25, 50 and 75 ppm) on plant growth, physiological parameters, yield and leaf nutrient status of strawberry cv. Chandler during the years 2013-14. The study showed significant effects of the treatments where the maximum plant height, leaf area, number of crowns per plant were recorded from T_{15}, while the maximum plant spread in T_{12} and the number of runners per plant in T_{18}. The number of fruits and yield were highest in T_{12} and lowest in control (T_{19}). The physiological parameters *viz*. chlorophyll content in T_9, rate of photosynthesis in T_{15} while stomatal conductance and transpiration rate in T_{18} were recorded maximum. Treatments also had significant effects on leaf nutrient contents in which maximum leaf nitrogen and manganese were recorded in T_9, whereas phosphorus, calcium, magnesium, zinc in T_{18}.The potassium was highest in T_6 and iron, copper in T_{12}. Study revealed that the PGPR can be used for sustainable fruit production.

Keywords: Strawberry, Growth, Leaf Nutrient, Physiological characters

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Introduction

Strawberry (*Fragaria x ananassa* Duch.), a temperate fruit crop has originated from the hybridization between *Fragaria chiloensis* L. P. Mill and *Fragaria virginiana* Duch. belonging to family Rosaceae. It is highly valued dessert fruit crop, popular for its taste flavor, rich source of vitamins (C,E,B), minerals, anti cancer properties and many other dietary compounds. In the early decades, the cultivation of strawberry was confined to temperate regions rendering it to price hiking. But due to its high cost return ratio, short growth phase and availability of new varieties, many farmers of states Punjab, Haryana, Delhi, Uttarakhand, J&K are looking forward for its further cultivation [1] and presently, it occupies an area of 0.5 thousand hectare with 3.5 thousand MT annual production in India [2], where Haryana is maximum strawberry producing state followed by Mizoram, Meghalaya, Maharashtra, Himachal Pradesh, Uttarakhand and Jammu & Kashmir. In Himachal Pradesh, it is being cultivated on 54 hectare with annual production of 84 MT [3]. Therefore, for increasing strawberry fruit production and quality, there is a great demand of improved management practices like use of plant growth promoting rhizobacteria and plant growth regulators in order to avoid excessive chemical use for high yield and quality and sustainable fruit production. Plant growth promoting rhizobacteria (PGPR) is defined as rhizosphere bacteria whose interaction with plants helps plant growth by supporting nutrition uptake, antagonizing or adding resistance to pathogens, production of plant hormones and modification of physical structure of the soil applications of PGPR in growth media or direct application to plant body during cropping are carried out to expect better yield as a result of plant/PGPR interaction. Positive effects of PGPR on commercial products have been reported in many crops [4-7]. Application of PGRs particularly GA_3 has been commercially recommended for cultivation of strawberry. Many research workers have endorsed application of GA_3 for strawberry cultivation [7]. Gibberellins are natural growth hormones which play primary role in stimulating auxin reaction that helps in controlling growth as well has direct effect on internode elongation, flowering, fruiting, quality and yield [1].

Material and methods

Experimental Detail

The research experiment was laid out as Randomized Block Design at Model Farm of Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan to monitor the effect of plant growth promoting rhizobacteria and GA_3 on strawberry cultivar Chandler during 2013-14. Five isolates of plant growth promoting rhizobacteria (S_1- *Bacillus licheniformis* CKA1, S_2- *Bacillus* subtilis CB 8 A, S_3- *Bacillus* sp. RG1, S_4 - *Bacillus* sp. S_1 and S_5 - *Bacillus* sp. S_2 @ 109 CFU each) in combination with GA_3 at 25, 50 and 75 ppm were used to treat the strawberry plants planted at a spacing of 50 x 25 cm during October, 2013 which resulted in nineteen treatments along with control *viz*. T_1- GA_3 @ 25 ppm, T_2- GA_3 @ 50 ppm, T_3 - GA_3 @ 75 ppm, T_4 - S_1 + GA_3 @ 25 ppm, T_5 - S_1 + GA_3 @ 50 ppm, T_6 - S_1 + GA_3 @ 75 ppm, T_7 - S_2+ GA_3 @ 25 ppm, T_8 - S_2+ GA_3 @ 50 ppm, T_9 - S_2 + GA_3 @ 75 ppm, T_{10} - S_3 + GA_3 @ 25 ppm, T_{11} - S_3+ GA_3 @ 50 ppm, T_{12} - S_3 + GA_3 @ 75 ppm, T_{13} - S_4+ GA_3 @ 25 ppm, T_{14} - S_4+ GA_3 @ 50 ppm, T_{15} - S_4 + GA_3 @ 75 ppm, T_{16} - S_5 + GA_3 @ 25 ppm, T_{17} - S_5+ GA_3 @ 50 ppm, T_{18} - S_5 + GA_3 @ 75 ppm and T_{19} - Control (sterile water application). Isolates and GA_3 were applied as foliar application method twenty days before expected flowering.

Parameters and Methods studied

Growth parameters

The effect of plant growth promoting rhizobacteria and GA_3 on growth was determined as plant height (cm) and spread (cm) which were measured with the help of a graduated scale while, the leaf area (cm^2) was recorded by leaf area meter (Licor-Model 3100). The number of crowns and number of runners per plant were recorded by counting their number from randomly selected five plants in each replication for each treatment and their average was expressed in numbers per plant. The number of fruits from randomly selected five plants were counted and weighed periodically at the time of harvesting and their average were worked out for determination of yield and expressed as grams/ plant.

Table-1 Plant growth promoting rhizobacteria and GA_3 affecting plant growth and yield

Treatment	Plant height (cm)	Plant spread (cm)	Leaf area (cm^2)	Number of crowns/ plant	Number of runners/ plant	Number of fruits / plant	Yield / plant (g)
T_1	26.6	32	112.07	3.78	19	18.89	355.82
T_2	27.43	33.42	113.67	4.16	19.88	20.78	443.87
T_3	29.17	39.92	116.56	4.7	23.08	21.56	484.52
T_4	27	36.92	114.94	4.38	24.53	22	486.1
T_5	28.67	38.67	118	4.78	25.42	22.44	511.42
T_6	29.58	41.5	122.68	4.8	30.92	23.67	570.71
T_7	28.2	38.83	113.88	4.17	20.74	20.89	403.86
T_8	29	40.17	118.67	4.5	26.58	21.55	471.77
T_9	30.7	40.92	126.93	4.74	29.85	23.11	512.52
T_{10}	27.33	41.67	112.53	4.12	19.37	19	459.36
T_{11}	29	46.25	114.67	4.4	22.75	23.44	467.95
T_{12}	30.67	47.17	118.63	4.74	23.97	24	586.57
T_{13}	27.83	39.92	128.2	4.11	25.21	20.89	472.7
T_{14}	28.17	41.67	133.31	4.54	26.92	22.22	549.41
T_{15}	31	43.75	137.95	5.17	32.09	23.78	513.97
T_{16}	26.67	40.42	121.2	4.33	21.73	19.67	443.51
T_{17}	28.33	41.5	124.73	4.78	24.98	20.56	459.5
T_{18}	29.42	43	137.74	4.88	33.71	22.78	488.48
T_{19}	24.88	31.63	110.2	3.6	18.42	15.78	267.09
$CD_{0.05}$	1.78	1.5	2.58	0.55	1.85	0.97	1.78

Table-2 Plant growth promoting rhizobacteria and GA_3 affecting physiological parameters

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	2.71	6.4	0.443	1.191	25.69
T_2	2.87	6.8	0.45	1.069	25.94
T_3	2.94	8.03	0.462	0.876	27.01
T_4	3.03	7.1	0.457	0.991	26.69
T_5	3.07	7.91	0.471	0.851	27.12
T_6	3.1	9.45	0.48	0.624	28.86
T_7	2.93	7.54	0.466	0.899	26.79
T_8	3.05	8.31	0.514	0.703	27.64
T_9	3.19	10.33	0.529	0.616	30.53
T_{10}	2.73	6.75	0.468	1.151	25.82
T_{11}	2.89	7.07	0.482	1.022	26.6
T_{12}	2.99	8.11	0.496	0.795	27.3
T_{13}	2.85	7.73	0.502	1.052	26.42
T_{14}	2.88	8.3	0.527	0.898	26.9
T_{15}	2.97	11.03	0.562	0.712	27.47
T_{16}	2.76	7.1	0.527	0.938	26.72
T_{17}	2.9	7.59	0.556	0.64	27.68
T_{18}	3.12	8.83	0.68	0.59	32.51
T_{19}	2.56	6.01	0.428	1.199	25.2
$CD_{0.05}$	0.1	0.2	0.041	0.061	0.77

Table-3 Plant growth promoting rhizobacteria and GA_3 affecting leaf nutrient status

Treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Zinc (ppm)	Iron (ppm)	Manganese (ppm)	Copper (ppm)
T_1	2.14	0.221	1.38	1.3	0.301	24.05	84.7	53.9	5.4
T_2	2.32	0.235	1.41	1.32	0.308	24.78	86.62	57.29	5.69
T_3	2.4	0.256	1.5	1.37	0.315	26.22	91.82	58.64	6.21
T_4	2.29	0.261	1.63	1.34	0.319	24.88	94.15	59.74	6.14
T_5	2.48	0.269	1.65	1.38	0.322	25.78	99.63	63.9	6.53
T_6	2.61	0.288	1.71	1.42	0.331	26.74	114.2	68.1	6.7
T_7	2.38	0.234	1.48	1.3	0.305	24.29	88.83	57.86	6.25
T_8	2.57	0.241	1.52	1.34	0.311	25.93	100.94	69	6.59
T_9	2.63	0.27	1.62	1.4	0.329	27	106.73	89.13	6.87
T_{10}	2.27	0.231	1.49	1.31	0.31	25.4	88.86	68.43	5.66
T_{11}	2.39	0.252	1.56	1.35	0.316	26.2	92.43	74	5.93
T_{12}	2.6	0.266	1.64	1.38	0.321	27.15	111.04	83.2	6.97
T_{13}	2.25	0.253	1.47	1.34	0.314	24.1	90.07	62.88	6.57
T_{14}	2.36	0.256	1.53	1.37	0.323	25.25	96.26	71.23	6.81
T_{15}	2.5	0.281	1.67	1.39	0.334	26.27	108.36	78.4	6.95
T_{16}	2.26	0.257	1.5	1.33	0.321	25.16	94.57	66.5	5.82
T_{17}	2.48	0.279	1.59	1.37	0.337	25.83	104.06	76.34	6.05
T_{18}	2.54	0.291	1.69	1.44	0.354	27.4	120.51	86.57	6.47
T_{19}	2.07	0.217	1.32	1.12	0.297	21.16	65.9	51.67	5.14
$CD_{0.05}$	0.1	0.02	0.12	0.08	0.041	1.05	1.32	1.05	0.28

Physiological parameters
The chlorophyll content (mg/g) was measured by Hiscox and Israelstam [8] while other physiological parameters (photosynthetic rate, stomatal conductance, stomatal resistance and transpiration rate) were recorded with the help of portable photosynthetic meter LICOR- 6200 and expressed as µ mol/m^2/s, m mol/s, S cm^{-1} and m mol/m^2/s.

Leaf nutrient status
The leaf nutrient contents of strawberry were determined by collecting fully expanded and matured leaf samples at the end of harvesting season and then drying, grinding and storing of samples were carried out as per procedure described by Kenworthy [9]. The leaf digestion for nitrogen estimation was carried out according to the procedure suggested by Jackson [10] and fed to Auto analyser Kjeltec Foss Tecator Model 2300 while for other nutrients, one gram leaf samples were digested in di-acid (HNO_3 and $HClO_4$ in 4:1 v/v) as suggested by Piper [11]. The leaf phosphorus was estimated by Vanado-molybdo-phosphoric acid method and potassium with the help of Flame photometer [10]. The Ca, Mg, Zn, Cu, Mn and Fe were carried out on Atomic absorption spectrophotometer model 4141.

Statistical Analysis
The method of analysis of variance as outlined by Gomez and Gomez [12] was used for statistical analysis of the data.

Results and Discussion
Growth and yield parameters
The observations made on plant growth characteristics [Table-1] showed significant effects as maximum plant height (31.00 cm) andleaf area (137.95 cm^2) were recorded from T_{15}, whereas the plant spread (47.17 cm) was maximum in T_{12}. The number of crowns per plant (5.17) were maximum in T_{15} followed by T_{18} (4.88) while the number of runners per plant (33.71) were highest in T_{18}. This significant effect on plant growth might be due to growth hormone producing efficiency of plant growth promoting rhizobacteria and GA_3 as it stimulates cell division and cell elongation in shoots and root. The results are in line with the work of several researchers who observed increase plant growth with the application of plant growth promoting rhizobacteria and plant growth regulators *viz*., Pandit *et al*. [13], Tripathi *et al*. [14], R Swamy *et al*. [15], Kumari and Mehta [7]. It is evident from the given data that fruit yield was also significantly increased with the application of plant growth promoting rhizobacteria in combination with GA_3@ 75 ppm as the highest number of fruits (24) and yield (586.57 g) per plant were recorded from the plant treated with plant growth promoting rhizobacteria in combination with GA_3@ 75 ppm (T_{12}).Similar findings were reported by Qureshi *et al*. [16], R Swamy *et al*. [15], Thakur *et al*. [17]. This increase in number of fruits and yield could be due to the development of differentiated inflorescence and formation of more metabolites by larger leaves as treated with plant growth promoting rhizobacteria and GA_3.

Physiological parameters
The given data [Table-2] reveals significant effect of treatments on physiological parameters that the application of plant growth promoting rhizobacteria in combination with GA_3 has resulted in enhanced physiological activity and showed increased chlorophyll content (3.19 mg/g) in T_9 significantly at par with T_{18} (3.12 mg/g) and rate of photosynthesis (11.03µ mol/m^2/s) in T_{15} while the maximum stomatal conductance (0.680 m mol/s), transpiration rate (32.51 m mol/m^2/s) and minimum stomatal resistance (0.590S cm^{-1}) were recorded in T18as compared to other treatments. This can be the due to the possibility of GA_3 and other growth hormones produced by plant growth promoting rhizobacteria resulting in increased leaf area, strong source sink relationship, better translocation of nutrients and water, increased nitrogen use efficiency and activities of nitrate reductase and carbonic anhydrase of plants. These results are in harmony with the findings of Misratia *et al*. [18] in rice, Moneruzzaman *et al*. [19] and Kumari *et al*. [6] in strawberry.

Leaf nutrients
The plant growth promoting rhizobacteria and GA_3 treatments had significant effects on leaf nutrient contents [Table-3] in which maximum leaf nitrogen (2.63%) and manganese (89.13 ppm)were recorded in T_9, whereas phosphorus (0.291%), calcium (1.44 %), magnesium (0.354 %), zinc (27.40 ppm) were recorded maximum in T_{18} as compared to control. The potassium (1.71 %) was highest in T_6 whereas iron (120.51 ppm) and copper (6.97 ppm) were highest in T_{12}. The findings are in accordance with work of Monge *et al*. [20] in peach, Shahin *et al*. [21] in apple and, Singh and Singh [22] in strawberry. Eid and Abou-Leila [23] also reported that GA_3 treatment increased the uptake of N, P, K, Mg, Fe, Zn, Mn and Cu content thereby increasing the mineral nutrient status of the plant. Enhancement in leaf nutrients by plant growth promoting rhizobacterial isolates and GA_3 could be attributed to increased photosynthetic activity, improved translocation of photosynthates and other metabolites to the sinks that might have contributed to the improved nutrient content of treated plants. The nutrient content as affected by GA_3 was also reported in 'Hass' avocado [24].

Conclusion
On the foregoing results, it may be inferred that different isolates of plant growth promoting rhizobacteria and GA_3 showed great potential to affect plant growth, yield, physiological activities and leaf nutrient content in strawberry. It further shows that their applications are safe, effective and can be easily adopted by growers for sustainable and ecological fruit production in order to reduce chemical fertilizers use.

Application of research: Study of the role of plant growth promoting rhizobacteria and GA3 in strawberry cv. Chandler has been reviewed and presented

Research Category: Plant growth regulator

Abbreviations: PGPR- plant growth promoting rhizobacteria, GA_3- gibberellic acid, ppm- parts per million, viz.-such as, cv.-cultivar, MT- metric ton, HNO_3- Nitric acid, $HClO_4$ - perchloric acid.

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University: Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, 173230, India
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Conflict of Interest: None declared

Sample Collection: Isolates of *Bacillus* sp. were collected from the Laboratory of Microbiology, Department of Basic Science, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, 173230, India

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number

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