DOI : 10.5958/0974-0112.2019.00064.1

Indian J. Hort. 76(3), September 2019: 400-404

Effect of PGPR on strawberry cultivation under greenhouse conditions

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ABSTRACT

The present study was conducted during 2016-18 to evaluate the effect of plant growth promoting rhizobacteria (PGPR) on strawberry cv. 'Chandler' under greenhouse conditions. Three PGPR strains (*Pseudomonas* sp. strain MHA75, *Bacillus* sp. strain RCA3 and *Bacillus* sp. strain SYB101) were used either alone or in combination as biofertilizer in a completely randomized design with four replicates. Data obtained from the study showed that the use of PGPR significantly increased fruit quality, yield, plant growth and control of disease. The inoculation of PGPR strains i.e., SYB101, MHA75 + SYB101, RCA3, MHA75 + RCA3 and MHA75 increased cumulative yield by 28.95, 28.15, 23.36, 18.60 and 17.46%, respectively. Ascorbic acid and anthocyanin content of fruit were increased significantly by the application of MHA75+ SYB101 (44.49, 45.54) ml/100 ml and SYB101 (43.96, 45.11) ml/100 ml as compared with the control (38.84, 40.43) ml/100 ml, respectively. Combinations of MHA75 + SYB101 and MHA75 + RCA3 showed 83.25 and 85.0% reduction in gray mould disease than control. Overall, the results of this study suggested that root inoculation with *Bacillus* sp. strain RCA3 and *Bacillus* sp. strain SYB101 alone or in combination with *Pseudomonas* sp. strain MHA75 have the potential to increase the yield and growth of strawberry. These microbial inoculants could be exploited for use under field conditions.

Key words: *Fragaria* × *ananassa, Bacillus*, *Pseudomonas*, gray mould disease, microbial inoculants.

INTRODUCTION

Plant-microbe interaction may occur at phyllosphere, endosphere and rhizosphere. Plant exudates such as amino acids and sugars provide a rich source of energy and nutrients for the bacteria in the rhizosphere, resulting in more microbial populations in this region than outside the region. Thus, rhizosphere has appeared as a versatile and dynamic ecological environment of intense plant-microbe interactions harnessing essential micro and macro nutrients affecting plant growth. In many rhizospheric relationships, PGPR are known to colonize the plant root and stimulate plant growth. The potentiality of PGPR in agriculture is steadily increased as it offers an attractive way to replace the use of chemical fertilizers, pesticides and other supplements. Plant growth is influenced by variety of stresses due to the soil environment, which is major constraint in sustainable agricultural production. Growth promoting substances are likely to be produced in large quantity by these rhizospheric microorganisms that indirectly influence the overall morphology of the plants (Bhattacharyya and Jha, 2). Various microorganisms such as *Azotobacter, Bacillus, Klebsiella, Azospirillum, Pseudomonas* and *Rhizobium* are being widely used as biofertilizers and biocontrol agents for reducing agrochemical inputs in agriculture and improvement in fruit production (Sindhu *et al*., 21). These bacteria provide fixed nitrogen and solubilized phosphorus/ potassium to the plants. Therefore, inoculation of agriculture/horticultural plants with these microbial inoculants has been found to improve the plant growth and yield of different agri-horti crops.

The strawberry (*Fragaria* × *ananassa* Duch.) being one of the most important species among berry fruits and its cultivated is gaining popularity being a highly valued economic fruit crop. In order to meet criteria of sustainable fruit production, Plant Growth Promoting Rhizobacteria (PGPR), are used as biofertilizers in place of synthetic chemicals, which improve plant growth through supply of plant nutrients and may help to sustain environmental health and soil productivity (O'Connell, 9). In recent years, use of PGPR as supplements to chemical fertilizers has been evidenced in the production of field, vegetable, forage and cash crops (Cvijanovic *et al*., 4). The present study was thus undertaken to explore the plant-microbe interaction (PGPR) on growth, yield, disease control, and quality of strawberry fruit.

MATERIALS AND METHODS

The study was conducted on strawberry cv. Chandler during two consecutive seasons of 2016-2017 and 2017-2018 under greenhouse conditions at CCS Haryana Agriculture University, Hisar, India (29º10' N latitude and 75º 46' E longitudes). The experiment was replicated twice in completely randomized design. The treatments contained

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inoculation with three biofertilizer strains, i.e., MHA75 (*Pseudomonas* sp.), SYB101 (*Bacillus subtilis*) and RCA3 (*Bacillus* sp.), whereas untreated soil served as un-inoculated control. Total 60 plants with four replications were inoculated with plant growth promoting rhizobacteria. The planting was done in the last week of October during the both years. Single uniform runner was planted in each pot after treating with carbendazim (0.1%). Each pot was filled with 4 kg of soil. Basal dose of fertilizers was added in each pot along with soil according to per plant requirement as per basal dose. Seventy five per cent fertilizers *viz*., urea, liquid NPK (19:19:19), SOP and MOP were given at weekly interval as per the recommendation.

Three PGPR strains i.e., MHA75 (*Pseudomonas* sp.), SYB101 and RCA3 (*Bacillus* sp.) were obtained from Dept. of Microbiology. These bacteria were reported as plant growth promoting bacteria and potential bio-control agents against a wide range of bacterial and fungal pathogens, and the beneficial attributes of these PGPR are listed in (Table 1). Bacterial cultures were maintained on Luria Bertani (LB) medium (Sambrook *et al*., 18) slopes.

Growth parameters such as height of plant was measured individually with a measuring scale, Plant spread was calculated by measuring the canopy of plant in East-West and North-South direction with the scale and the average of both was expressed as plant spread, Number of leaves per plant was counted from the time of transfer to the end of growing season (November - March) at fortnightly interval and the average number of leaves per plant, crown diameter of plant was measured with the help of fruit dimensions (mm) (length and breadth) were also determined in the samples by 'Inox' vernier scale (±0.05 mm accuracy), Fresh weight of plant recorded at after harvesting of fruits with the help of electronic balance METTLER balance (±0.01 g accuracy) and plants taken for fresh weight were first dried at room temperature and there after dried in oven at 45°C for five to six days until the reduction in weight became constant.

Yield parameter such as fresh weight of fruits in each replication were randomly selected to determine average fruit weight using METLER balance (±0.01 g accuracy) and the data were expressed in g per fruit. Fruit dimensions (mm) (length and breadth) were also determined in the samples by 'Inox' vernier scale (±0.05 mm accuracy). Quality traits, *viz*., TSS, acidity, ascorbic acid, anthocyanin content and moisture content of fruit were measured at commercial maturity stages. TSS (%) was determined using hand refractometer having a range of 0 - 32 (ERMA) by putting a drop of juice and taking the readings. The titratable acidity (%) and ascorbic acid (ml/ 100 ml) was determined as per the method suggested by AOAC (1) and anthocyanin content (ml/100 ml) was determined using pH differential method. The moisture content (%) of fruits taken for fresh weight were first dried in oven at 55°C up to 15 days until the reduction in weight became constant. The disease control was calculated on visual basis.

Before planting of strawberry, baseline soil properties of soil samples were recorded. Soil samples were air dried, crushed and passed through a 2-mm sieve prior to chemical analysis. The Kjeldhal method (Bremner, 3) was used to determine total N. Plant- available P was determined by using sodium bicarbonate method (Oleson *et al*., 10). Soil pH was determined in 1:2 extracts, and EC were determined according to (Jackson, 5). Soil organic carbon was determined using Walkley and Black rapid titration method (Piper, 17). Flame photometric method (Jackson, 6) was used to determine available K. Based on soil analysis and the mean values, the experimental soil was found to be sandy in texture, low in organic carbon, available nitrogen and phosphorous and available potash content were medium in the soil.

The data was subjected to analysis of variance (ANOVA) using OP STAT statistical computer package (Sheoran, 20). The Critical Difference (CD) was used to compare treatment means and treatments declared different at p = 0.05 level of significance., pooled data was used for discussion purpose.

RESULTS AND DISCUSSION

Two years of experimental trials (during 2016 and 2018) showed that inoculation of rhizobacterial strains affected plant height, plant spread, number of leaves,

Table 1. Beneficial characteristics of PGPR isolates used for inoculation.

Bacterial cultures	Relevant characteristics					Reference for source
	ALA (µg/ml)	IAA (µg/ml)	**HCN	***P	*ACC	
Bacillus strain SYB101	1.16	2.77	-	+	++	Phour and Sindhu, 14
Pseudomonas strain MHA75	7.94	3.73	-	+	-	Khandelwal *et al*., 7
Bacillus strain RCA3	10.42	8.11	+++	+++	+++	Sehrawat, 7

*Growth on ACC incorporated plates indicates ACC deaminase activity, which reduces the level of stress hormone ethylene.**HCN production contributes in disease control. ***P solubilization increase phosphorus availability in soil.

crown diameter, fresh weight and dry weight of plants. The results showed that inoculation with PGPR strains i.e., MHA75 + SYB101, SYB101, MHA75 + RCA3, RCA3 and MHA75 treatment significantly affected all the growth parameters, *viz*., plant height, spread, number of leaves, crown diameter, fresh weight and dry weight of plant compared to control (Table 2). The most significant enhancement in plant height, spread and number of leaves per plant were obtained by inoculation with MHA75 + SYB101 (13.35 cm, 23.67 cm and 11.92) followed by strain SYB101 (13.21 cm, 23.52 cm and 11.79) treatment as compared to control. Crown diameter, fresh and dry weight of plants was significantly increased by application of strains MHA75 + SYB101 (13.02 mm, 44.49 g and 12.85 g) as compared to control.

In general, combination of *Pseudomonas* strain sp. MHA75 and *Bacillus* strain sp. SYB101 and *Bacillus* strain SYB101 were found more effective compared to other applications in terms of growth, yield quality and quality as compared to the control. Similar findings were reported in previous studies showing that application of PGPR strains may stimulate growth and yield parameters in chrysanthemum (Kumari *et al*., 8). Many PGPR strains have been identified as having the ability to produce the plant growth regulators indole-3-acetic acid (IAA) and/or other plant hormones α- aminolevulinic acid (ALA) in the rhizosphere, which play an important role in plant growth promotion and yield (Phour *et al*., 15).

This study showed that rhizobacterial treatments significantly affected the fruit yield and number of fruits per plant (Table 3). Significant yield increase was obtained by inoculation with *Bacillus* sp. strain SYB101 (242.30 g/plant), MHA75 + SYB101 (240.81 g/plant), *Bacillus* sp. strain RCA3 (231.82 g/plant), MHA75 + RCA3 (222.86 g/plant) and *Pseudomonas* sp. strain MHA75 (220.71 g/plant) as compared with the control (187.91 g/plant) (Table 3). The percentage of yield increase was 28.95%, when *Bacillus* sp. strain SYB101 was inoculated. However, no significant differences were found between treatments concerning the average berry weight and fruit properties such as fruit size, acidity and moisture content, except TSS, ascorbic acid and anthocyanin content. Similar to yield improvement, inoculations with *Bacillus* sp. strain SYB101, RCA3 and MHA75 + SYB101 increased the number of fruits per plant (22.25, 22.0 and 21.75) compared to control (19.25), respectively. TSS, ascorbic acid and anthocyanin content in fruits were significantly affected by treatment of rhizobacterial strains compared to control (Table 4). TSS content in fruits were found 7.45, 7.44, 7.43, 7.32 and 7.21% in RCA3, MHA75, MHA75 + SYB101, SYB101 and MHA75 + RCA3 respectively, inoculated with rhizobacterial strains than un-inoculated control. On the other hand, highest ascorbic acid (44.49 mg /100ml) and anthocyanin content (45.54 ml/100 ml) of fruit was obtained in MHA75+ SYB101 inoculated plants. It was further observed that rhizobacterial treatment significantly controlled the gray mould disease in fruits of strawberry (Table 4). The minimum disease incidence was observed on fruits of plants inoculated with rhizobacterial strain MHA75 + RCA3 (15%) compared to control (24%).

Earlier studies reported that all the three rhizobacterial isolates used in this study produced growth promoting hormones IAA and ALA, and solubilized bound phosphorus (Phour and Sindhu, 14). ACC utilization was observed in isolates SYB101 and RCA3, and isolates RCA3 also produced HCN to kill the pathogens (Phour and Sindhu 14; Sehrawat, 19; Khandelwal *et al*., 7). It is well known that PGPR strains that produce plant hormones can stimulate plant cell elongation or cell division and/or change bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Patten and Glick, 11), which prevent the production of plant growth-inhibiting hormone, ethylene (Penrose *et al*., 12). In addition, exogenous application of IAA has been found to contribute to colonization efficiency and to the growth and survival of PGPR on host plants (Vandeputte *et al*., 22). Moreover, the presence of high numbers of bacteria in the rhizosphere is important in order to

Table 2. Growth promoting effect of rhizobacterial isolates on strawberry cv. Chandler.

Observations/ Treatments	Control	RCA 3	MHA75	SYB101	MHA75 + RCA3	MHA75+ SYB101	CD (0.05%)
Plant height (cm)	11.8	12.73	12.47	13.21	12.89	13.35	0.17
Plant spread (cm)	21.44	23.12	23.22	23.52	23.31	23.67	0.21
Number of leaves per plant	9.87	11.34	11.54	11.79	11.69	11.92	0.26
Crown diameter (mm)	11.44	12.66	12.47	12.86	12.78	13.02	0.12
Fresh weight of plant (g)	40.11	43.35	42.72	43.86	43.79	44.49	0.31
Dry weight of plant (g)	10.71	12.13	11.88	12.62	12.34	12.85	0.15

Table 3. Effect of inoculation of rhizobacterial strains on yield of strawberry.

Observations/ Treatments	Control	RCA 3	MHA75	SYB101	MHA75 + RCA3	MHA75 + SYB101	CD (0.05%)
Days to flower	89	83	81	83	82	80	NS*
Number fruit per plant	19.25	22	21	22.25	20.5	21.75	0.21
Fruit weight (g)	9.86	10.8	10.52	10.91	10.87	11.07	NS*
Length of fruit (mm)	38.89	40.63	40.16	40.72	40.7	41.17	NS*
Width of fruit (mm)	27.81	29.11	28.67	29.27	29.19	29.33	NS*
Yield per plant (g)	187.91	231.82	220.71	242.3	222.86	240.81	3.25

*NS non significant

Table 4. Effect of rhizobacterial strains on quality and gray mould incidence on fruit of strawberry.

Observations/Treatments	Control	RCA3	MHA75	SYB101	MHA75 + RCA3	MHA75 + SYB101	CD (0.05%)
TSS (%)	6.31	7.45	7.44	7.32	7.21	7.43	0.08
Acidity (%)	0.84	0.73	0.74	0.76	0.77	0.8	NS
Ascorbic acid (ml /100ml)	38.84	42.34	42.24	43.96	43.53	44.49	0.51
Anthocyan content (ml /100ml)	40.43	43.89	43.97	45.11	44.51	45.54	0.76
Moisture content (%)	94.07	92.78	92.86	92.56	92.68	92.49	NS*
Gray mould disease incidence (%)	24	18	21	20	15	16.75	1.24

*NS non significant

convert insoluble forms of organic substances into available plant nutrients (Pii *et al*., 16), which can affect the vegetative growth of strawberry plants. Therefore, one of the possible mechanisms by which PGPR inoculation has enhanced the growth and yield of strawberry plants in this study may be due to the production of plant growth regulators and increasing the availability of nutrients similar to findings reported in earlier study by Pesakovic *et al.,* (13).

The study revealed that *Bacillus* strains SYB101 and RCA3 and/or *Pseudomonas* strain MHA75 either alone or in combinations were found to have great potential for use as PGPR to increase the production of strawberry. Moreover, these PGPR strains could also suppress gray mould disease caused by *Botrytis cineraria* in strawberry. From the present study the recommendation can be made that chemical fertilizers can be substituted by biofertilizers which not only improve the yield but also obtain health and environment-safe products. Further studies are required under field conditions for application of these PGPR as biofertilizers and biopesticides for sustainable agriculture.

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Received : June, 2019; Revised : August, 2019;
Accepted : August, 2019