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Microbial Biofertilization to Improve Yield and Quality of Strawberry

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Abstract: Microorganisms inhabiting the soil and rhizosphere take part in biogeochemical cycles and enhance the availability of macronutrients leading to improved soil productivity and enhanced food production. This study was conducted to examine the effect of inoculation of plant growth-promoting Rhizobacteria (PGPR) on phenological data, yield per plant and fruit quality characteristics of strawberry (*Fragaria x ananassa* Duch) cv. 'Chandler' during 2016 and 2017. Root inoculations with rhizobacteria significantly increased yield per plant (1.59–25.03%) and average fruit weight (1.00-12.86%) compared to control, whereas the bacterial inoculations did not affect dry weight of plant, fruit size and moisture content in strawberry cv. 'Chandler'. The bacterial inoculations also increased soluble solid content (SSC),ascorbic acid and anthocyanin content in strawberry. The ascorbic acidand anthocyanin contents of fruits ranged from 38.70 ml /100ml (control) to 41.41 ml/ 100ml (CPS67), 39.90 ml/ 100ml (control) to 42.96 ml/ 100ml (CPS67), while TSS varied between 6.26% (control) and 7.45% (HMM57). Thus, inoculation ofrhizobacterial strains JMM15, HMM57, HMM92 and CPS67 have the potential to increase the yield and growth of strawberries and could provide a promising sustainable strategy to improve strawberry growth in low fertility soils.

Keywords: Strawberry, PGPR, Root inoculation, Growth, Yield

Strawberries (*Fragaria × ananassa* Duch.) are unique in their highly desirable taste and flavor. Most of its cultivars are octaploid with 2n= 56. Its fruits are rich in bioactive phytochemicals especially phenolic compounds with high antioxidant capacity. It also contains vitamins and minerals making the daily diet beneficial to human health. In India, the cultivated area under strawberry is mainly located in Maharashtra, Himachal Pradesh, Uttar Pradesh, Haryana and the Nilgiri hills is nearly 1,000 ha with a production of 5,000 tonne, and in Haryana, is around 150 ha with a production of 2,010 tonne (Rao and Saxena 2017). The modern strawberry cultivation that warrants high yield and quality requires extensive use of chemical fertilizers, which not only disrupt the balance of nature but also reduce economic efficiency. The continuous use of chemical fertilizers not only affects soil health and environment adversely but also reduces the productivity of crops. This situation emphasized the need for the development of alternate production systems, which are environment friendly and is more judicious to manage the soil health. More recently, the use of plant growth promoting rhizobacteria (PGPR) has been exploited in fruit crops (Sindhu et al 2010) and proved a major tool in fruit production. Plant growth promoting rhizobacteria may improve plant growth and yield by means of producing plant growth regulators (auxin, gibberellins, cytokinins), solubilizing/mineralizing the organic phosphate or other nutrients, fixing atmospheric nitrogen and facilitating the uptake of nutrients (Sharma et al 2018, Sindhu and Sharma 2019). Various research workers found that plant growth promoting rhizobacteria could stimulate plant growth and increase yield of apple, sweet cherry, citrus, raspberry, high bush blueberry, mulberry and apricot. Inoculation of PGPR species could increase the growth attributes like leaf area, dry weight of both the root and aerial parts of the inoculated plants, resulting in better development. The application of PGPR strains (*Pseudomonas* BA-8, *Bacillus* OSU-142 and *Bacillus* M-3) through roots enhanced the quality of strawberry fruits by increasing the total soluble solids, total sugars and reducing sugars significantly, and decreasing the titratable acidity (Pirlak and Kose 2009). PGPRs like *Bacillus*, *Pseudomonas*, *Azotobacter* and *Azospirillum* promote the synthesis of growth promoting substances like auxins, gibberellins, cytokinins and antibiotic metabolites, which in turn improve resistance against biotic and abiotic stresses (Tomic et al 2015). The plant rhizosphere bacteria of genera *Pseudomonas* and *Bacillus* have been recognized as early root colonizers, which enhance plant growth by increasing seed emergence, plant weight and crop yield. The environmental benefits of this innovative approach reduces the use of agro-chemicals and fitting with sustainable management practices. Recent progress in understanding the biological interactions that occur in the rhizosphere and the need for inoculants formulation and delivery may facilitate its commercial development (Mohanram and Kumar 2019). The objective of this study was to investigate the growth-promoting effects of PGPR strains inoculation under greenhouse conditions in relation to phenological data, total yield and fruit quality characteristics of strawberry.

MATERIAL AND METHODS

Study site and planting material: The study was conducted on 'Chandler' strawberry plants during the consecutive seasons (2016-2017 and 2017-2018) in greenhouse at CCS Haryana Agriculture University, Hisar (29º10' N latitude and 75º 46' E longitudes). The experiment was replicated twice in completely randomized design. The treatments contained four fertilizer types, *Pseudomonas* strains namely, JMM15, HMM57, HMM92 and CPS67 (containing 10^8 bacterial cell/ml), whereas untreated soil served as 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 to soil in each pot along according to per plant requirement as per basal dose. The seventy five per cent fertilizers *viz.,* urea, liquid NPK (19:19:19), sulphate of potash and murate of potash were given at weekly interval as per the recommendation.

Treatments: Four PGPR strains HMM57, HMM92, JMM15 and CPS67were 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 for different cereal and oil seed crops (Table 1). Bacterial cultures were maintained on Luria Bertani (LB) medium slopes (Sambrook et al 1989).

Estimation of IAA, ALA, salt tolerance and phosphate solubilization: Rhizobacterial isolates were tested for their ability to produce indole acetic acid using Salkwoski's Reagent (Gordon and Weber 1951). Indole acetic acid (100 µg/ml) was used as standard and results were expressed as µg IAA produced per ml of culture supernatant. Uninoculated LB broth served as control. For α-aminolevulinic acid isolates were tested using the method as described by Mauzerall and Granick (1955). The concentration of ALA in the culture supernatant of different rhizobacterial isolates was determined by using standard curve. Screening of rhizobacterial isolates for growth at different salt concentrations: selected rhizobacterial isolates were checked for their ability to grow at different concentrations of NaCl upto 8% (w/v) on LB medium containing 20 Mm HEPES (N-2- hydroxyethane- sulphonic acid) buffer (Marsudi et al 1999). Medium plates were spotted with 20 µl growth suspension of different bacterial isolates. The plates were incubated for 3-4 days at 28±2°C in a BOD incubator. The susceptibility or tolerance to NaCl was recorded by observing the growth as a positive or negative result and colony size was measured. Phosphorus solubilization by rhizobacterial isolates was studied on Pikavskaya's medium plates by spot test method (Sharma et al 2011). Phosphorus solubilization by different rhizobacterial isolates were scored based on their ability of solubilization zone formation.

Growth, yield and fruit quality traits: 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 'Inox' vernier scale (±0.05 mm accuracy), Fresh weight of plant recorded at after harvesting of fruits and plants taken for fresh weight were 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 fresh weight of ten fruits in each replication were randomly selected to determine average fruit weight and the data were expressed in g per fruit. Fruit dimensions (mm) (length and breadth) were also determined in the samples. Quality traits, viz., TSS, acidity, ascorbic acid, anthocyanin content and moisture content of fruits were measured at commercial maturity stages. Total soluble solids (%) 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/ 100ml) was determined as per the method suggested by AOAC (1990) and anthocyanin content (ml/ 100 ml) was determined using pH differential method. The moisture content (%) of fruits was taken for fresh weight. Then fruits were dried in oven at 55°C up to 15 days until the reduction in weight became constant.

Statistical analysis: The data was subjected to analysis using OP STAT statistical computer package (OP Sheoran 2004, CCS HAU Hisar).

RESULTS AND DISCUSSION

Phosphate-solubilizing capacities, salt tolerance and production of IAA and ALA by PGPR strains: All the rhizobacterial strains showed IAA and ALA production (Table 1). Significant phosphate solubilization was observed in JMM15 and HMM92 strains. All the rhizobacterial strains showed salt tolerance upto 8% NaCl concentration.

Effect of PGPR inoculation on the yield, growth and fruit quality: Root inoculation of strawberry plants with different rhizobacterial strains showed significant effect on the growth parameters, *i.e.,* plant height, number of leaves, plant spread, fresh weight and dry weight of plant, except dry weight of plants (Table 2). All the growth parameters increased significantly with the application of *Pseudomonas* strain CPS67 in association with 75% RDF. Wani et al (2007) also reported that *Pseudomonas* spp. secrete organic acids and enzymes tha tact as mineralization of immobile form of phosphates and also produces amino acids, vitamins and growth-promoting substances, resulting in promotion of plant growth. The quality of fruits in terms of total soluble solids, ascorbic acid and anthocyanin content increased significantly with the application of different *Pseudomonas* strains in strawberry plants.

The ascorbic acid content and anthocyanin content (Table 3) was recorded in fruits of strawberry plants inoculated with *Pseudomonas* strain CPS67 with lowest value for acidity in fruits, whereas maximum TSS was recorded in strain HMM57. Anuradha et al (2020) recorded maximum TSS and ascorbic acid in strawberry fruits harvested from the plants inoculated with biofertilizers. Biofertilizers increased the accumulation of carbohydrates, which converted into disaccharides leading to higher sweetness. Moreover, root inoculation of strawberry plants with *Pseudomonas* strain CPS67 had significantly positive effect on the yield parameters, *i.e.,* number of fruits per plant, fresh weight of fruit and yield of fruit, except size of fruits (length and breadth). The maximum number of fruits per plant, fresh weight of fruits and yield of fruits per plant was obtained in plants inoculated with *Pseudomonas* strain CPS67 (Table 4). The number of fruits per plant was the highest in CPS67 treatment (20.75) and followed by HMM57 (20) and control (18.75). The highest fruit weight was in treatments having inoculation with *Pseudomonas* strain CPS67 (10.44 g) followed by HMM57,JMM15, HMM92 and control (Table 4). In brief, the bacterial inoculation increased the fruit weight by 1.00-12.86% compared to control. The bacterial treatments increased yield per plant by 1.59-25.03% compared to control. All the bacterial strains revealed higher yield values than the control that varied from 176 to 216 g in bacterial treatments.

The bacterial strain CPS67 was most effective treatments for growth, yield and quality among all treatments. Statistical differences were not recorded for dry weight of plant, acidity, moisture content and fruit size between any tested bacterial strain and control. The present study showed that root inoculation with CPS67 and HMM57 promoted plant growth and yield of Chandler strawberry cultivar, but growth responses were strain-specific. The positive effects of PGPR application on growth, yield and quality of apricot, raspberry, tomatoes,, sugar beet, apple, sweet cherry and barley were explained by promoting abilities of these bacteria for auxin and cytokinin production, N_2-fixation, phosphate solubilization and antimicrobial substance production

Table 1. Beneficial characteristics of PGPR isolates

Bacterial cultures	Relevant characterstics				Reference for source
	ALA (µg/ml)	IAA (µg/ml)	***P	Salt tolerance 8%	
JMM 15	17.45	12.24	+++	+++	Phour, 2016
HMM 57	11.74	4.62	-	++	Phour, 2016
HMM 92	17.53	0.99	+++	+++	Phour, 2016
CPS 67	7.5	50.25	+	++	Phour, 2012

Table 2. Effect of plant growth promoting rhizobacteria on growth traits of strawberry plants

Treatments	Plant height (cm)	Plant spread (cm)	Number of leaves per plant	Crown diameter (mm)	Fresh weight of plant (g)	Dry weight of plant (g)
JMM 15	11.35	20.58	9.94	11.12	40.13	10.58
HMM 57	11.54	21.27	10.27	11.30	40.96	11.07
HMM 92	11.28	20.46	9.69	11.03	39.83	10.50
CPS 67	12.24	22.73	11.18	12.19	42.54	11.72
Control (75%)	11.20	20.22	9.49	10.86	39.44	10.39
100 %	13.82	23.18	11.85	12.83	45.00	12.73
CD (p=0.05)	0.21	0.46	0.23	0.16	0.63	NS

NS= non significant

Table 3. Effect of plant growth promoting rhizobacteria on quality parameters of strawberry fruits

Treatments	TSS (%)	Acidity (%)	Ascorbic acid (ml/ 100ml)	Anthocyanin content (ml /100ml)	Moisture content (%)
JMM 15	6.77	0.84	40.18	41.01	94.23
HMM 57	7.45	0.81	40.57	41.50	93.95
HMM 92	6.44	0.83	39.49	40.48	94.28
CPS 67	7.34	0.75	41.41	42.96	93.21
Control (75%)	6.26	0.86	38.70	39.90	94.43
100 %	7.57	0.72	45.03	46.75	92.58
CD (p=0.05)	0.08	NS	0.64	0.79	NS

NS= non significant

Table 4. Effect of plant growth promoting rhizobacteria on fruit size and yield of strawberry plants

Treatments	Fresh weight of fruit (g)	Length of fruit (mm)	Breadth of fruit (mm)	Number of fruits	Yield per plant (g)
JMM 15	9.46	38.31	27.34	19.25	182.11
HMM 57	9.71	38.60	27.61	20.00	196.10
HMM 92	9.34	38.18	27.24	18.88	176.20
CPS 67	10.44	39.88	28.54	20.75	216.84
Control (75%)	9.25	37.91	27.06	18.75	173.43
100 %	10.90	40.74	29.17	22.87	243.05
CD (p=0.05)	0.18	NS	NS	0.31	3.21

NS= non significant

(Cakmakci et al 2001, Esitken et al 2006, Orhan et al 2006, Pirlaket al 2007, Aslantus et al 2007, Karlidag et al 2007). The yield and growth enhancement effects of bacteria used in this study on strawberry could be explained by similar reasons.

Rhizobacterial isolates used in this study were analyzed for growth promoting hormones IAA, ALA and solubilize bound phosphorus (Phour and Sindhu 2019).The hormones of auxin group have positive effect on fruit setting in different fruit species. In our study, higher yield obtained by bacterial inoculation of strawberry plants may therefore be explained by IAA producing capacity of strain CPS67. The presence of high number of bacteria in the rhizosphere is also important since they may mineralize bound form of nutrients to plant utilizable form.

CONCLUSION

The beneficial activities of PGPR inoculants in the rhizosphere of inoculated plants may provide promising solutions for sustainable and environment-friendly agriculture. The different rhizobacterial strains in association with 75% RDF were found to have great potential to increase the production of strawberry. From the present study' the recommendation can be made that application of chemical fertilizers may be reduced by inoculation of biofertilizers strains which not only improve the yield but also provide to obtain health and environment-safe products. Further studies are required under field conditions for application of these PGPR as biofertilizers and for sustainable agriculture.

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