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Bio-inoculation of strawberry plants with *Bacillus strains* having promoting effect on growth, yield and quality

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Abstract

The quality of agricultural production and their modulation by biofertilizer application has gradually received attention. However, the importance of *Bacillus* strains has not been studied extensively especially in strawberry. The present investigation was carried out to study the effect of *Bacillus* strains on growth, yield and quality of strawberry *cv.* Chandler during the year 2016-17 and 2017-18. The study indicates that various *Bacillus* strains significantly influenced growth, yield and quality of fruits. Among different *Bacillus* strains, HCA61 strain recorded the significantly highest fruit yield per plant (233.69 g). It also recorded the highest value concerning different growth parameters, *viz.* plant height (12.56 cm), spread (22.78 cm), and number of leaves per plant (11.31), crown diameter (12.46 mm), fresh weight (43.86 g) and dry weight (12.36 g) of plant with a minimum number of days (79.50 days) to flower in strawberry plants. Among different yield attributing characters like number of fruits per plant (22.13), fresh weight of fruit (10.56 g), fruit length (40.31 mm), fruit breadth (28.83) and moisture content in fruit (92.83%), HCA61 strain showed superiority than other *Bacillus* strains. Regarding various physico-chemical characters, *viz.* TSS, acidity, ascorbic acid and anthocyanin content of fruits, *Bacillus* RCA3 strain exhibited significantly maximum values. The *Bacillus* strain HCA61 caused improved growth and yield. However, concerning physico-chemical characteristics of fruits *Bacillus* RCA3 strain was found best. The results indicated that inoculation with *Bacillus* strains could be an eco-friendly and cost-effective technology for improving strawberry growth, yield and quality.

Key words: *Bacillus*, strawberry, total soluble solids, yield

Introduction

Strawberry (*Fragaria × ananassa* Duch.), belonging to the family Rosaceae, is known as one of the most attractive, delicious and refreshing fruits of the world and occupies an important place among the orchard plants. In India, the cultivated area under strawberry is mainly located in Maharashtra, Himachal Pradesh, Uttar Pradesh, Haryana and the Nilgiri hills, having nearly 2,000 ha area with a production of 5,000 tonnes. In Haryana, it is around 150 ha, producing 2,010 tonnes (NHB, 2021). Strawberry crop emerged as a hub, especially near the NCR region of the capital, due to the availability of its market in Delhi and its quick considerable income and nutritional values. The modern strawberry cultivation that warrants high yield and quality requires extensive use of chemical fertilizers, disrupting the balance of nature and reducing economic efficiency. This situation emphasized the need to develop alternate production systems, that are environment friendly and more judicious to manage soil health (Sindhu *et al.,* 2018). Strawberry is a shallow rooted herbaceous plant, of which 50-90% of the root system is confined to the upper 15 cm soil (Sakamoto *et al.,* 2016). Bio fertilizers, more appropriately called microbial inoculants, are preparations containing live or latent cells of efficient strains of microorganisms (Saini *et al.,* 2017). These biofertilizers are cost-effective environment-friendly inputs that play a crucial role in reducing the inorganic fertilizer application. Biofertilizers are important components of integrated nutrient management (INM) and their use as bioinoculants is most beneficial for establishing different horticultural species in a rain-fed ecosystem (Mir and Sharma, 2012). Considering these facts, there is a pressing need to study the inoculation of strawberry plants with *Bacillus* promoting the growth, yield and quality of strawberry.

Materials and methods

The experiment was conducted at a Hi-tech greenhouse and in the Department of Horticulture Postharvest laboratory, CCS Haryana Agricultural University, Hisar (Haryana), between 2016-17 and 2017-18. A semi-arid climate with hot and dry summers and cold winters characterizes the Hisar region. The soil of the experimental plot was sandy in texture, low in organic carbon, with soil pH 7.82; the available N, P and K were 95, 10 and 102 kg ha^{-1}. The experiment was laid out in Complete Randomized Design (CRD) with 11 treatments and four replications. The recommended dose of P_2O_5 and K_2O @ were applied during pot filling. The various treatments followed for the investigation were as follows: T_0 =75% RDF + MSA39, T_1 =75% RDF +SYB101, T_2 = 75% RDF + SB153, T_3 = 75% RDF + JMM24, T_4 = 75% RDF + HMM39, T_5 = 75% RDF + HCA61, T_6 = 75% RDF + RCA3, T_7 = 75% RDF + RCA8, T_8 = 75% RDF + HCA76 (*Bacillus* control), T_9 = 75% RDF and T_{10} = 100% RDF. Seventy-five percent of the recommended fertilizer dose was applied after strawberry runners were planted in the respective pots, as mentioned in the treatments. Observations on various growth and yield characters were recorded by using standard methods. Ten berries from each treatment were randomly selected to record the data on physico-chemical characters. Crown diameter and fruit size were recorded by measuring length and breadth using the digital Vernier callipers, whereas fruit weight was taken using top pan digital balance. Total soluble solids were determined with a Zeiss hand Brix refractometer (0- 32°B). The method, as suggested by Anon. (1990), was used to determine the titratable acidity and ascorbic acid. Anthocyanin content was measured using pH differential method. The data was subjected to analysis of variance (ANOVA) using OP STAT statistical computer package (Sheoran, 2004). The Critical Difference (CD) was used to compare treatment means at $P = 0.05$ level.

Results and discussion

Effect of *Bacillus* strains on growth characteristics of strawberry: Root inoculation of strawberry plants with different *Bacillus* strains showed a significant effect on all the growth parameters, *i.e.,* plant height, number of leaves, plant spread, appearance of the first flower, fresh weight of plant, dry weight of plant and number of runners per plant, but growth responses were strain specific. The 100% RDF produced the highest growth and yield of fruits but among the *Bacillus* strains, the plant height (12.80 cm), plant spread (22.80 cm), leaves per plant (11.31) and crown diameter (12.46 mm) increased significantly with the application of strain HCA61 with 75% RDF (Table 1). The increase in plant height, number of leaves, plant spread and crown diameter with *Bacillus* inoculation might be since biofertilizer promotes root development due to the production of hormones and solubilization of nutrients, which results in better vegetative growth (Pii *et al.,* 2015; Sindhu *et al.,* 2010). Earlier, Aslantas *et al.* (2007) and Karlidag *et al.* (2007) also reported increased growth of apple with application of *Bacillus* strains. Plant growth-promoting rhizobacteria increased plant growth by improving soil physical, chemical and biological properties, which provided a better environment for nutrient uptake by the plants. The earliness of flower appearance in plants inoculated with *Bacillus* strain HCA61 might be due to the easy uptake of nutrients and simultaneous transport of growth-promoting substances like auxins and cytokinins to the axillary buds, breaking apical dominance. Ultimately, this has resulted in a better sink for faster mobilization of photosynthates and early transformation of plant parts from the vegetative to the reproductive phase (Verma and Rao, 2013). The outcomes of the current experiment align with Ipek *et al.* (2014) findings, which indicated a correlation between floral induction in strawberries and the presence of cytokinin, polyamines, and bacteria. Specifically, the bacteria, known for producing auxin, may have contributed to an increase in plant root growth. Kumari *et al.* (2014) stated that application of plant growth promoting rhizobacteria reduced the days taken for the appearance of first flower in chrysanthemum. The study indicated a significant increase in fresh and dry weight and runners of strawberry plants inoculated with *Bacillus* strain HCA61 than the un-inoculated ones (Table 1). The improvement in fresh and dry weight and runners of plants might be due to the increased availability of nutrients for growth and production of phytohormones by the rhizobacteria, which prolonged the vegetative growth and, therefore, increased photoassimilates.

The present results align with the findings of Awasthi *et al.* (1998), who related the increased plant growth to the general role of biofertilizers in stimulating nutrient uptake, especially nitrogen, which played an important role in the assimilation of numerous amino acids. Enhanced amounts of amino acids and proteins contributed to the weight of the plant. The increased number of runners per plant might be due to increased plant growth in height, number of leaves and crown diameter, which accumulated more photosynthates, increasing the number of runners per plant in strawberry (Tripathi *et al.,* 2016). Similar results were also reported by Lufti and Murat (2009) and Gupta and Tripathi (2012).

Moreover, rhizobacteria increased the availability of nutrients to the plants and their translocation from root to flower through plant foliage (Singh and Singh, 2009). Applications of biofertilizers have been reported to play an important role in enhancing plant growth and yield through a wide variety of mechanisms. Many PGPR strains have been identified that can produce plant growth substances such as indole-3-acetic acid, cytokinin and other plant hormones in the rhizosphere, which are important in promoting plant growth and yield (Malik and Sindhu, 2011).

Effect of *Bacillus* strains on yield and yield attributing characters of strawberry: Various *Bacillus* strains significantly influenced the yield and yield-attributing characteristics of strawberries. It is revealed from Table 2 that maximum yield was obtained in HCA61 strain treatment, while minimum in control (75% RDF). The increased yield of plants inoculated with *Bacillus* strain HCA61 might be due to the increased number of berries per plant, length, width, and weight.

It is revealed from the data that variation on number of berries per plant was significant. Berry length (40.31 mm), berry diameter (28.83 mm) and berry weight (10.56 g) were also influenced significantly by *Bacillus* strain HCA61 than other treatments. Erturk *et al.* (2012) reported that strawberry plants inoculated with RC 19 (*Bacillus simplex*) gave the highest number of berries and

Table 1. Effect of *Bacillus* strains on growth characteristics of strawberry

Treatments	Plant height (cm)	Plant spread (cm)	Leaves/ plant	Crown diameter (mm)	Emergence of first flower (days)	Fresh weight of plant (g)	Dry weight of plant (g)	Runners / plant
T_0	11.48	21.08	10.20	11.66	86.25	40.58	11.10	5.25
T_1	12.56	22.47	11.05	12.24	82.50	42.98	12.10	7.00
T_2	11.65	21.32	10.36	11.81	83.75	41.37	11.37	5.63
T_3	11.35	20.62	9.99	11.37	87.00	40.29	10.85	5.13
T_4	11.30	20.49	9.74	11.22	87.63	39.98	10.66	5.00
T_5	12.80	22.78	11.31	12.46	79.50	43.86	12.36	7.00
T_6	12.22	22.17	10.77	12.06	83.63	42.38	11.83	6.25
T_7	11.95	21.95	10.52	11.89	84.00	41.89	11.58	6.00
T_8	11.28	20.34	9.62	10.96	88.63	39.81	10.47	4.63
T_9	11.23	20.25	9.53	10.88	90.00	39.46	10.42	4.50
T_{10}	13.75	23.14	11.87	12.81	75.63	44.96	12.70	8.50
CD = (0.05%)	0.25	0.28	0.24	0.17	1.89	0.59	0.21	0.24

Table 2. Effect of *Bacillus* on yield and yield attributing characters of strawberry

Treatments	Berry length (mm)	Berry dia. (mm)	Berries/ plant	Fresh weight of berry	Yield/ plant
T_0	38.52	27.67	20.25	9.71	196.62
T_1	39.96	28.69	21.75	10.4	226.85
T_2	38.77	27.88	20.75	9.82	203.76
T_3	38.34	27.50	19.50	9.52	185.64
T_4	38.16	27.32	19.25	9.41	182.15
T_5	40.31	28.83	22.13	10.56	233.69
T_6	39.69	28.43	21.75	10.31	224.24
T_7	39.21	28.11	21.25	10.10	214.62
T_8	38.07	27.20	19.00	9.31	178.89
T_9	37.93	27.09	18.87	9.27	177.09
T_{10}	40.72	29.17	22.75	10.87	243.05
CD = (0.05%)	0.18	0.20	0.51	0.18	4.86

yield, which might be due to production of IAA and cytokinin. Tripathi *et al.* (2015) also reported that the increase in berry size and weight might be due to the increased photosynthetic ability of plants, which in turn might have favoured and increased the accumulation of dry matter. Berry weight and size are highly correlated with dry matter content and balanced level of hormones. Among all the treatments, *Bacillus* strain HCA61 recorded the highest yield (233.69 g/ plant). The lowest yield (177.09 g/plant) was recorded in control. The results of the present experiment corroborate the findings of Verma and Rao (2013), who reported that the increase in berry yield and its parameters might be due to an increase in the number of leaves, which worked as an efficient photosynthesis structure and produced a high amount of carbohydrates in the plant system. Moreover, the capability of rhizobacteria to produce growth hormones, enzymes, and antifungal and antibacterial compounds may have contributed to increased fruit yield in strawberries (Sharma *et al.,* 2018).

Effect of *Bacillus* strains on quality parameters of strawberry fruit: Data presented in Table 3 revealed that different *Bacillus* strains had a significant role in the physico-chemical characteristics of berries. The highest TSS (7.44 %), lowest acidity (0.74%) and highest anthocyanin content (45.00 mg/ 100 ml) were recorded in berries inoculated with *Bacillus* strain RCA3. The increased TSS in berries might be due to the availability of assimilates due to increased photosynthesis and nutrient availability in fruit plants due to the application of plant growth-promoting bacteria

Table 3. Effect of *Bacillus* strains on quality parameters of strawberry fruit

Treatments	TSS (°Brix)	Acidity (%)	Ascorbic acid (mg/ 100 mL)	Anthocyanin content (mg/ 100 mL)	Moisture content (%)
T_0	6.78	0.81	40.63	42.03	93.94
T_1	7.31	0.78	43.69	44.00	92.95
T_2	7.30	0.80	41.53	42.52	93.63
T_3	6.55	0.84	40.34	41.47	94.07
T_4	6.51	0.83	39.66	40.92	94.13
T_5	7.37	0.76	42.86	44.06	92.83
T_6	7.44	0.74	42.22	45.00	93.15
T_7	7.09	0.79	40.97	42.83	93.46
T_8	6.35	0.85	39.33	40.42	94.30
T_9	6.25	0.86	38.69	39.90	94.43
T_{10}	7.57	0.72	45.01	46.75	92.60
CD= (0.05%)	0.11	NS	1.03	1.17	0.15

(Thakur *et al.,* 2015). The decreased acidity in fruit juice might be due to the metabolic transformation of organic acids into sugars and the rapid utilization of organic acids in respiration (Esitken *et al.,* 2010). The ascorbic acid content (43.69 mg/ 100 mL) in strawberry fruits was highest when the plants were inoculated with *Bacillus subtilis* strain SYB101. The respective increase in ascorbic acid and anthocyanin content might be due to the increased efficiency of microbial inoculants to fix atmospheric nitrogen and secrete growth-promoting substances, which accelerated physiological processes like carbohydrate synthesis (Tripathi *et al.,* 2016).

Correlation coefficient values of growth and yield attributing character of strawberry: Data presented in Table 4 and 5 revealed the correlation between the growth parameters and the yield of strawberry. The estimates for the correlation coefficient for plant height, plant spread, leaf number, crown diameter and yield in all possible combinations are presented in Table 4. All the growth and yield attributing characters showed a highly significant positive correlation with yield. A highly significant positive association with yield was shown by plant spread (r = 0.998), and other traits, *viz.* plant height (r = 0.942), leaf number (r = 0.981), and crowns diameter (r = 0.985) also showed a highly positive correlation with yield. Similarly, the yield positively correlated with yield attributing characters of berries, *viz.* berries plant^{-1}, berry weight, length and diameter (Table 5). The berries plant^{-1} (r = 0.942), berry weight (r = 0.998), berry length (r = 0.981) and berry diameter (r = 0.985) were highly positively correlated with yield and effect was found to be highly significant for all the parameters.

Table 4. Correlation coefficient values betweem growth characters and yield of strawberry

Trait	Plant height	Plant spread	No. of leaves	Crown diameter	Yield
Plant height	1	0.947	0.978	0.946	0.942
Plant spread		1	0.982	0.984	0.998
No. of leaves			1	0.991	0.981
Crown diameter				1	0.985
Yield					1

Table 5. Correlation coefficient values betweem yield attributing berry traits and yield of strawberry

Trait	Berry/ plant	Berry weight	Berry length	Berry diameter	Yield
Berry/plant	1	0.991	0.979	0.987	0.996
Berry weight		1	0.996	0.997	0.996
Berry length			1	0.996	0.991
Berry diameter				1	0.993
Yield					1

From the results of the present investigation, it can be concluded that the *Bacillus* strain HCA61 was the most efficient strain for improving the growth and yield of 'Chandler' strawberry. The physico-chemical characteristics of fruits were improved with inoculation of *Bacillus* strain RCA 3. Hence, these two *Bacillus* strains can be recommended for inoculating strawberry to obtain enhanced growth, yield and quality of strawberry. Thus, beneficial rhizobacterial strains could be used as bio fertilizers in the integrated nutrient management (INM) strategy for sustainable agriculture.

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