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Effect of Foliar Spraying with Pink Pigmented Facultative Methylotrophic Bacteria on the Growth and Productivity of Strawberry

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Abstract: Pink-pigmented facultative methylotrophic bacteria (PPFM) isolated from cotton leaves was identified based on phenotypic and genotypic characteristics as *Methylobacterium rodiotolerance*. Two field experiments were conducted to investigate the effects of PPFM, methanol (10 and 30%) and a combination of bacteria and methanol on the growth, fruit quality, and yield of two strawberry cultivars (Florida and Festival). The main differences between the two cultivars are greater foliage fresh weight and early yield in cv. Florida while Festival cv. had a higher total yield per plant and greater anthocyanins and ascorbic acid contents. The greatest vegetative growth, dry matter percentage, potassium content, and carbohydrate content besides the earliest yield per plant were observed following spraying with PPFM or PPFM mixed with 10% methanol. Spraying with PPFM resulted in the highest total yield per plant, highest yield per feddan, and fruit quality. Spraying cv. Florida with PPFM resulted in the best early yield while spraying cv. Festival with PPFM resulted in the highest total yield and fruit quality. Spraying with PPFM appears to be the most efficient treatment for enhancing the total yield of Festival cv. by an average of 23.02 and 24.06 tons per feddan in the first and second seasons, respectively.

1 Introduction

Strawberry (*Fragaria* x *ananassa* Duch.) is a major fruit crop worldwide due to its nutritional value, health benefits, and demand as an export. Strawberry has high natural antioxidant and flavonoid content and has been shown to have anti-cancer effects (Hossain et al 2016). Strawberry has become one of Egypt's most significant horticultural crops for fresh consumption, processing, and exports. According to FAOSTAT, strawberries ranked fifth among exported crops in Egypt in 2020 with a planted area of approximately 26,756 feddan producing 433,945 tons and 36,939 tons of exports in the 2020–2021 season.

Pink-pigmented facultative methylotrophic bacteria (PPFM) are a type of aerobic, gram-negative bacteria that can live on one-carbon compounds including formate, formaldehyde, and methanol (Abanda-Nkpwatt et al 2006). Methylobacterium sp. functions as a biofertilizer, a biocontrol agent, and a producer of plant growth regulators such as auxins and cytokinins which

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promote plant growth Nadali et al (2010). Methylobacterium sp. affects physiological plant processes including the production of enzymes that control plant growth such as urease and 1-aminocyclopropane-1-carboxylatedeaminase Madhaiyan et al (2006). Methanol is a naturally occurring by-product of pectin metabolism in plant cells, with a proportion of produced methanol discharged into the environment while the rest is converted to formaldehyde, formic acid, and CO_2. The release of CO_2 affects the efficiency of CO_2 absorption in plants and encourages the synthesis of secondary metabolites (Galbally and Kirstine 2002). Methanol-derived CO_2 can also be utilized by PPFM (McTaggart et al 2015). PPFM has a considerable impact on the growth of numerous crops including tomatoes (Subhaswaraj et al 2017), potatoes (Grossi et al 2020), and ginger (Vadivukkarasi and Bhai 2020). Methanol is a carbon source that Methylobacterium sp. is able to use for both growth and energy (Vadivukkarasi and Bhai 2020). Accordingly, the present study aimed to evaluate the effects of foliar spraying with pink-pigmented facultative methylotrophic bacteria and methanol on the development and production of two strawberry cultivars.

2 Material and Methods

2.1 Isolation and purification of PPFM

Young cotton leaves collected from the garden of the botany department at the Faculty of Women for Arts, Science, and Education were used for the isolation of PPFM. Approximately 3–4 young leaves were washed in tap water to remove debris and then dried and imprinted on the surface of ammonium mineral salt (AMS) medium supplemented with 0.5% methanol as a sole carbon source (Whittenbury et al 1970). All plates were incubated at 30°C for one week. Following adequate growth, PPFM colonies were selected based on their characteristic pink color and purified on AMS medium before being stored on AMS slants at 4°C for short-term storage or as 30% glycerol stock at 80°C for long-term storage.

2.1.1 Identification of PPFM

The Methylobacterium isolate used in the present study was identified based on biochemical tests in Bergey's Manual of Systematic Bacteriology (Garrity et al 2015). Molecular identification was performed using 16SrRNA analysis (Jayashree et al 2011). Established bacterial cultures in AMS broth were centrifuged at 4000 rpm for 10 min. DNA extraction was performed using Gen Jet genomic DNA purification kits (Thermo K0721). Total extracted DNA was used as a template and amplified by PCR using a forward primer 8F: (5-AGAGTTT-GATCCTGGCTCAG-3) and reverse primer 1520R: (5-TGCGGCTGGATCACCTCCTT-3) to obtain a product of approximately 260 bp.

2.1.2 16S rDNA sequencing and data analysis

Sequencing analysis was performed on a 255 bp PCR product. Sequence analysis was performed using the ABI 3130 genetic analyzer and Big Dye Terminator version 3.1 cycle sequencing kits. The 16S rRNA sequence was aligned and compared with other partial 16S rDNA gene sequences in GenBank using the NCBI Basic Local alignment search tools BLAST-n program (http://www.ncbi.nlm.nih.gov/BLAST). Multiple alignments of sequences and assessments of nucleotide sequence statistics and variability were performed using CLUSTALW (ver. 1.74) (Thompson et al 1994). Egyptian Methylobacterium isolates were compared to other isolates registered in NCBI using Molecular Evolutionary Genetics Analysis software (ver. 4.0) (Tamura et al 2007).

2.1.3 Inoculum preparation

PPFM isolates were grown on MS medium (Whittenbury et al 1970) for ten days in an orbital shaker at 120 rpm and 30°C. Bacterial growth was evaluated using a spectrophotometer and found to be 2 OD at 600 nm (Madhaiyan et al 2006). Stock solutions were further diluted for plant colonization by combining 10 mL with 1 liter of distilled water.

2.2 Field experiment

Field experiments were conducted at EL-Qanater Research Station Farm in Qalubia Governorate, Egypt, over the 2017/2018 and 2018/2019 seasons using freshly transplanted strawberry cv. Festival and Florida. Transplants were obtained from the Strawberry and Non-Traditional Crops Improvement Center of Ain Shams University's Faculty of Agriculture. Transplants were planted on the 1st and 4th of October in the two growing seasons, respectively. The experimental soil was clay loam with a pH of 7.67 and EC of 0.82 ds/m^{-1}. Three equal applications of ammonium sulfate (20.5% N) at 60 kg per feddan, potassium sulfate (48% K2O) at 50 kg per feddan, and calcium super phosphate (15.5% P2O5) at 45 kg per feddan were applied 30 and

45 days after planting. Weather conditions at the research station during the experimental period according to the Central Laboratory for Agricultural Climate are shown in **Table 1**. Fresh transplants were grown in raised beds 20–50 cm high and 100 cm wide in furrows 30 cm apart using a drip irrigation system. The experiment was conducted using a split-plot design with three replicates and a 20 m^2 plot size with two cultivars in the main plot and six randomly assigned foliar spraying treatments in the subplot as follows: 1) distilled water; 2) 10% methanol; 3) 10% methanol; 4) PPFM and 10% methanol; 5) PPFM and 30% methanol; and 6) PPFM only.

Spraying began one month after planting and was repeated six times at three-week intervals. As the effect of methanol depends on a relatively low air temperature in the morning, both the upper and lower leaf surfaces were sprayed until moist (Rajala et al 1998).

Table 1. Average air temperature, relative humidity %, and rainfall during the 2017/2018 and 2018/2019 seasons

Months	2017/2018 Season		
	Average air Temperature (°C)	Relative humidity (%)	Rain (mm)
October	22.58	54.61	6.70
November	17.17	65.33	84.50
December	14.35	69.52	1.60
January	11.88	70.82	28.80
February	15.34	58.48	5.50
March	19.06	47.30	0.70
April	21.58	45.68	39.10
May	26.64	42.00	0.20
2018/2019 Season			
October	26.4	50.8	4.80
November	20.5	55.7	10.9
December	15.1	63.3	9.20
January	12.55	50.1	2.2
February	14.1	51.7	7.70
March	16.4	51.5	11.00
April	20.35	43.3	12.8
May	26.93	32.13	0.00

2.2.1 Measurement of growth parameters

2.2.2 Vegetative growth characteristics

At the beginning of flowering, data were collected from a random selection of 10 plants from each experimental plot. Plant height was measured in cm and total leaf area (cm^2) was measured using a CI-202 USA laser area meter. The fresh weight of foliage was recorded and calculated as dry matter weight divided by the total foliage weight. Leaf chlorophyll was measured using a handheld chlorophyll meter (SPAD–502, Konica Minolta Sensing, Inc., Japan).

2.2.3 Fruit yield and composition

The early yield per plant was calculated using the weights of all harvested fruits during the first four harvests. Total yields per plant and per feddan were measured from the first harvest to the first week of June. Fruit quality was determined by randomly selecting 30 fully developed fruits from each treatment group in the middle of the growth season (March in both seasons). Fruit firmness was measured using a Chatillon penetrometer. A digital refractometer model (ATAGO N-20E) was used to calculate the proportion of total soluble solids (TSS). Titratable acidity and vitamin C content were measured according to the methods of the Association of Official Agricultural Chemists (AOAC 2005). The total anthocyanin content fruit was determined according to the methods of Rangana et al (1979). At 120 days after planting, the total amount of carbohydrates in crowns was determined according to the methods of James et al (James et al 1995). The potassium content (%) in leaves was determined using a Flame Photometer (AOAC, 2016). The presence of the PPFM strain on the stomata of young plant leaves was confirmed using scanning electron microscopy (JEOL JSM 5200, JEOL Technics Ltd., Japan).

2.3 Statistical analyses

The means of different treatment groups were compared using analysis of variances with the least significant differences computed according to Snedecor and Cochran (1991). P-values less than 0.05 were considered statistically significant.

3 Results and Discussion

A PPFM was isolated from the young leaves of a cotton plant on MS medium supplemented with 0.5% methanol as a sole carbon and energy source using the leaf impression technique. The isolate was identified as a PPFM based on its characteristic pink color (Madhaiyan et al 2005). The isolate was pink-pigmented, aerobic, gram-negative, catalase-positive, oxidase-positive, urease-positive, Voges-Proskauer (VP)-positive, motile, and nitrate-reducing. In addition, the isolate had a negative result in the indole and methyl red test. The isolate was unable to utilize starch or gelatin as carbon sources. These results are in accordance with the phenotypic characteristics of the Methylobacterium genus described by Madhaiyan et al (2005).

Partial 16S rRNA sequence analysis indicated that the strain belongs to *Methylobacteria* with 100% similarity to *Methylobacterium radiotolerance* accession number, MT644122.1 (**Fig 1**).

3.1 Characteristic vegetative growth

3.1.1 Plant height

No significant difference in plant height was observed between cv. Florida and cv. Festival (**Table 2**). Foliar spraying with PPFM bacteria separately or combined with 10% or 30% methanol produced the plants with the greatest heights in both seasons. However, the lowest values for plant height were recorded in controls. Spraying cv. Florida with 30% methanol and cv. Festival with PPFM bacteria mixed with methanol at 10% in the first season had the greatest effects on plant height. The maximum interaction value was recorded for the spraying of cv. Florida with PPFM bacteria mixed with 10% methanol during the second season. Methanol may stimulate the expression of pectin methyl esterase, thereby increasing the amount of pectin in cell membranes. Higher pectin levels lead to increased production of methanol and CO_2 availability and promote plant tissue elongation and expansion (Ramírez et al 2006). Moreover, treatment with PPFM bacteria alone or combined with 10% methanol increased the plant height of both cultivars in both seasons. This finding may be attributable to the bacterial production of phytohormones such as auxin that promote plant cellular division, extension, and elongation.

3.1.2 Leaf area

No significant difference in leaf area was observed between the two cultivars (**Table 2**). Spraying strawberry plants (both Festival and Florida cultivars) with PPFM combined with 10% methanol resulted in the greatest leaf area. Spraying with PPFM had no effect on the leaf area in either season. The greatest effect on leaf area in both seasons was observed with the spraying of strawberry cv. Florida with PPFM mixed with 10% methanol. These findings may be attributable to methylotrophic bacteria utilizing volatilized methanol released through stomata as carbon and energy sources to produce growth-promoting substances such as auxin and cytokinin which play important roles in the regulation of plant growth and development (Kutschera 2007). Methanol foliar spraying has been shown to decrease leaf senescence by increasing the duration of active photosynthesis and leaf area (Glick et al 1998). Further, methanol foliar spraying has important effects on dominant cell expansion by activating cell wall synthesis (Armand et al 2016, Poornimmal 2020).

3.1.3 Fresh foliage weight

Florida cv. plants had greater fresh foliage weight compared to Festival cv. Strawberry. Plants treated with PPFM had the greatest fresh foliage weight values in both seasons (**Table 2**), corroborating the results of Nadali et al (2010). Florida plants treated with PPFM or PPFM mixed with 10% methanol resulted in the greatest increase in fresh foliage weight in the first season. These findings are consistent with the results of Abbasian et al (2016). The addition of methanol to Methylobacteria may have a positive effect on increasing CO_2 levels in leaves resulting in increased photosynthetic production and delayed leaf senescence by preventing ethylene-mediated growth suppression (Glick et al 1998).

3.2 Early yield

Florida plants had greater early yield compared to cv. Festival plants in both growing seasons (**Table 3)**. These results may be due to genetic differences between the two cultivars, as mentioned by Chandler et al (2009). The highest early yield was achieved by PPFM alone or in combination with 10% methanol in both seasons. Spraying Florida plants with PPFM alone produced the highest early yields, with no significant difference observed between 30% methanol and 30% methanol combined with PPFM in the first season. In the second season, PPFM alone or combined with 10% methanol resulted in the highest early yields. The combination of Methylobacteria with methanol may increase the amount of methanol consumed by Methylobacteria, thereby increasing plant phytohormone synthesis and promoting host growth via the release of metabolites (Abanda-Nkpwatt et al 2006). Similarly, methanol has been shown to improve the growth and yield of marigold plants (Khalilzadeh and Pirzad 2021).

3.3 Total yield

Festival plants had a higher total yield per plant than cv. Florida plants in both growing seasons (**Table 3)**. Furthermore, no significant difference in total yield per feddan was observed between the two cultivars in the first season; however, Festival plants had a higher

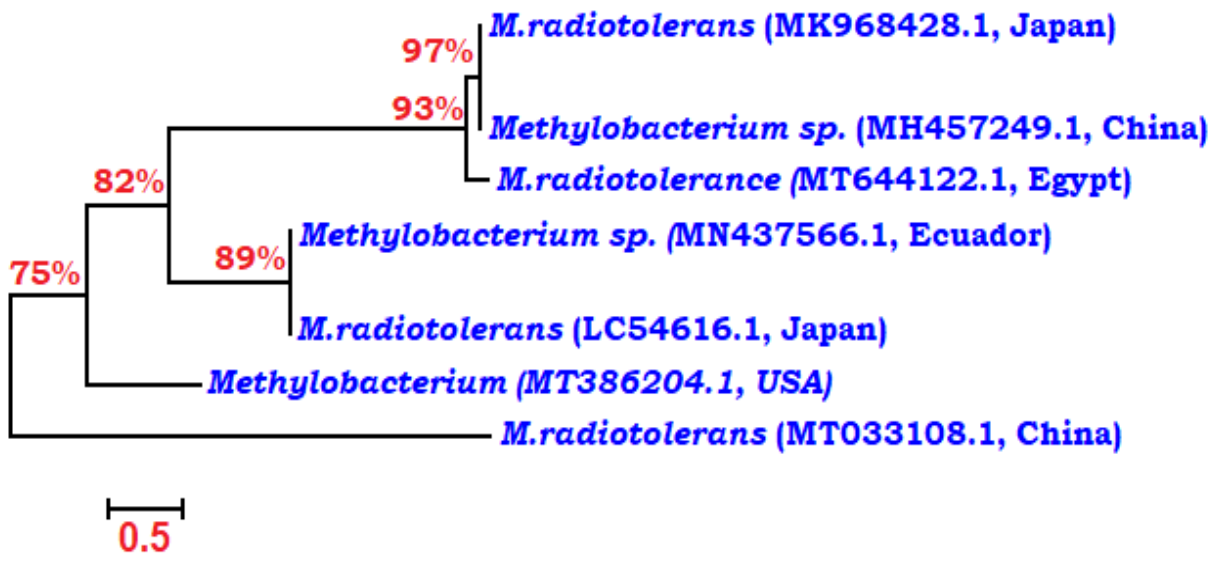

Fig 1. Phylogenetic tree of the *Methylobacterium* isolate based on 16S rRNA sequence analysis showing its relationship with neighbors. Bootstrap values greater than 70% for 1000 replicates are shown at branch note. Bar, 0.5%.

Table 2. Effect of spraying with *Methylobacterium*, methanol, or a combination of *Methylobacterium* on the growth of two strawberry cultivars during the 2017/2018 and 2018/ 2019 seasons

Characters	Plant height (cm)			Leaf area (cm^2)			Foliage fresh weight (g)		
Cultivars / Treatment	Florida	Festival	Mean	Florida	Festival	Mean	Florida	Festival	Mean
2017-2018 Season									
Control	20.19 f	19.27 f	**19.7C**	783.3ef	673.6 g	**728.5 D**	53.53 f	34.68 l	**44.1D**
10% methanol	22.27e	23.67 cd	**22.9 B**	976.6 b	848.7d	**912.7 B**	60.89 e	42.05 j	**51.7 C**
30% methanol	24.83 a	23.03 de	**23.93 A**	862.3 cd	785.1 e	**823.7 C**	63.18 d	40.43 k	**51.81 C**
PPFM + 10% methanol	23.73 bcd	24.80 a	**24.3 A**	1031. a	886.6 c	**958.8 A**	65.71 b	46.12 h	**55.9AB**
PPFM + 30% methanol	24.20 abc	22.30 e	**23.3 B**	871.7 cd	758.4 f	**815.0 C**	64.95 c	45.34 i	**55.2 B**
PPFM	23.97 abc	24.63 ab	**24.30 A**	987.1 b	857.1 d	**922.1 AB**	66.62 a	46.98 g	**56.8 A**
Mean	**23.2 A**	**22.95 A**		**918.7 A**	**801.6 A**		**62.48 A**	**42.60 B**	
2018-2019 Season									
Control	20.63 f	22.17 e	**21.40 C**	797.4 d	686.9 e	**742.2D**	58.16e	37.51 k	**47.84 E**
10% methanol	24.70 d	24.93 d	**24.82 B**	998.9 b	864.1 c	**931.5 BC**	65.84d	45.53 i	**55.68 C**
30% methanol	26.63 ab	25.83 c	**26.23 A**	876.2 c	866.5 c	**871.4 C**	66.83 c	42.78 j	**54.81 D**
PPFM + 10% methanol	26.90 a	26.00 bc	**26.45 A**	1121 a	991.2 b	**1056.1 A**	71.56 a	49.52 h	**60.54 B**
PPFM + 30% methanol	24.97 d	25.83 c	**25.4AB**	1005 b	875.6 c	**940.2BC**	68.92b	51.21g	**60.1 B**
PPFM	26.10 bc	26.37 abc	**26.23 A**	1049 b	1003 b	**1026 AB**	71.93 a	52.11f	**62.02 A**
Mean	**25.07 A**	**25.31 A**		**974.6 A**	**881.2 A**		**67.21 A**	**46.44B**	

Values within columns or rows followed by the same capital or small letter do not significantly differ from each other according to Duncan's multiple range test at 5% significance.

Table 3. Effect of spraying with *Methylobacterium*, methanol, or a combination of *Methylobacterium* on the yield of two strawberry cultivars during the 2017/2018 and 2018/ 2019 seasons

Characters	Early yield (g/plant)			Total yield (g/plant)			Total yield (ton/fed)		
Cultivars / Treatments	Florida	Festival	Mean	Florida	Festival	Mean	Florida	Festival	Mean
2017-2018 Season									
Control	190.7 d	130.9 e	**160.8 C**	426.2 l	473.8 k	**450.0 E**	16.98 l	18.95 k	**17.97 E**
10% methanol	224.8 c	195.6 d	**210.2 B**	510.5 j	559.4 e	**534.9 D**	20.42 j	22.31 e	**21.36 D**
30% methanol	235.2 bc	227.7 c	**231.4 AB**	515.2 i	569.1 c	**542.2 C**	20.60 i	22.76 c	**21.68 C**
PPFM + 10% methanol	239.9 abc	231.9 bc	**235.9 AB**	548.7 g	594.8 b	**571.7 B**	21.88 g	23.79 b	**22.84 B**
PPFM + 30% methanol	251.9 ab	240.9 abc	**246.4 AB**	528.9 h	561.9 d	**545.4 C**	21.12 h	22.48 d	**21.80 C**
PPFM	257.9 a	244.0 abc	**251.0 A**	552.4 f	598.9 a	**575.6 A**	22.08 f	23.96 a	**23.02 A**
Mean	**233.4 A**	**211.8 B**		**513.6 B**	**559.6 A**		**20.51 A**	**22.37 A**	
2018-2019 Season									
Control	192.6 e	142.2 h	**167.4 D**	452.6 k	488.1 j	**470.4 F**	18.10 k	19.52 j	**18.81 F**
10% methanol	231.4 c	169.7 g	**200.5 C**	538.3 h	571.8 f	**555 E**	21.52 h	22.87 f	**22.19 E**
30% methanol	235.2 bc	174.1 g	**204.7 BC**	535.6 i	582.1 d	**558.8 D**	21.43 I	23.27 d	**22.35 D**
PPFM + 10% methanol	255.3 a	192.5 e	**223.9 A**	570.6 f	620.8 b	**598.5 B**	22.85 f	24.99 b	**23.92 B**
PPFM + 30% methanol	239.1 b	184.2 f	**211.7 B**	546.9 g	588.3 c	**567.6 C**	21.88 g	23.50 c	**22.69 C**
PPFM	259.3 a	200.7 d	**230.0 A**	578.5 e	626.8 a	**602.7 A**	23.05 e	25.07 a	**24.06 A**
Mean	**235.5 A**	**177.2 B**		**537.4 B**	**580.3 A**		**21.47 B**	**23.21 A**	

Values within columns or rows followed by the same capital or small letter do not significantly differ from each other according to Duncan's multiple range test at 5% significance.

total yield in the second season. These results may be due to differences in climatic conditions between growing seasons (**Table 1**). Treatment with PPFM resulted in the highest total yield per plant and total yield per feddan in both seasons followed by PPFM mixed with 10% methanol. Festival plants treated with PPFM had the greatest increase in total yield per plant and per feddan in both seasons. This increase may be attributed to PPFM producing phytohormones, providing nutrients, and inducing defense responses against plant pathogens. Further, the excretion of methanol from growing plants may promote the colonization of PPFM as observed in potato plants by Ardanov et al (2016).

3.4 Phytochemical properties

3.4.1 Chlorophyll content

No significant differences in chlorophyll content were observed between the two cultivars in either season (**Table 4**). The spraying of strawberry plants with PPFM resulted in the highest chlorophyll content in both seasons. Methylobacterium has been shown to improve photosynthetic activity by increasing stomatal count and chlorophyll content in rice plants (Aswathy et al 2020). Spraying with methanol has increased beneficial effects when combined with Methylobacteria (Armand et al 2016). Florida plants sprayed with PPFM had the greatest increases in chlorophyll content in both

seasons followed by the spraying of Festival plants with PPFM in the second season.

3.4.2 Dry matter content of foliage

No significant differences in the proportion of dry matter in the foliage was observed between cv. Florida and cv Festival (**Table 4**). Spraying strawberry plants with PPFM or PPFM mixed with 10% methanol resulted in the highest proportion of dry matter in foliage. Furthermore, spraying with PPFM combined with 30% methanol or methanol alone resulted in a significant increase in dry matter content compared with controls in both seasons. The increased dry matter is associated with hormonal balance as PPFM synthesizes a range of auxins and cytokinin that are used by host plants for development (Koenig et al 2002). In Florida plants, spraying with PPFM resulted in the greatest increases in foliage dry matter in the first season followed by spraying of Festival plants with PPFM mixed with 10% methanol. These results are consistent with those obtained by El Badawy et al (2017) in strawberries and Valizadeh-Kamran et al (2019) in marigold and lavender plants.

3.4.3 Carbohydrate content of strawberry crowns

No significant differences in carbohydrate content were observed between the two strawberry cultivars (**Table 4**). Foliar spraying with PPFM alone or combined with 10% methanol resulted in the highest carbohydrate content in both seasons. This finding may be attributable to PPFM promoting photosynthesis and reducing photorespiration by increasing the concentration of CO_2 around stomata, thereby increasing carbohydrate synthesis (Ramírez et al 2006). Foliar application of methanol at 10% and 30% had a positive impact on carbohydrate content compared to the control in both seasons. Similar results were obtained by Omran et al (2009) who found that methanol provided the greatest increase in the carbohydrate content of Flame seedless grapevines. In addition, PPFM provided the greatest increase in the carbohydrate content of Florida plants. These results may be due to the effect of PPFM on promoting plant development by increasing auxin and cytokinin levels while inhibiting ethylene synthesis.

3.5 Physicochemical characteristics

3.5.1 Fruit firmness

No significant difference in fruit firmness was observed between the two cultivars in either season (**Table 5**). PPFM treatment resulted in the greatest fruit firmness, with no significant differences observed between 10% methanol and PPFM combined with 30% methanol in either season. This finding may be attributable to the effects of methanol on increasing cell wall strength resulting in delayed plant senescence and reduced ethylene production, thereby increasing plant growth and fruit quality (Ramírez et al 2006). Further, PPFM treatment of Festival plants resulted in the greatest effect on fruit firmness, with no noticeable difference observed after treatment with 10% methanol or PPFM combined with 30% methanol in either season, a finding also reported by El Badawy et al (2017).

3.5.2 TSS content of fruit

No significant difference in total soluble solid content was observed between the two cultivars (**Table 5**). PPFM, 30% methanol, and combined treatment with PPFM and 30% methanol resulted in a higher total soluble solid content compared to the control in both seasons. This result may be due to the utilization of methanol at plant stomata for photosynthesis, resulting in increased sugar production (Nadali et al 2010). Further, these treatments had the greatest effect on Florida plants in both seasons. In Festival plants, PPFM alone or PPFM combined with 30% methanol resulted in the highest TSS values.

3.5.3 Fruit acidity

There was no significant difference in acidity content between cv. Florida and cv. Festival fruits in the two growing seasons (**Table 5**). In the first season, PPFM treatment resulted in a lower fruit acidity content compared to PPFM and PPFM mixed with methanol in the second season. Ardanov et al (2016) and Zabetakis (1997) reported that colonization of strawberry plants with Methylobacteria may improve the biosynthesis of certain compounds that affect strawberry fruit quality. The lowest acidity interaction values were observed for Florida plants sprayed with PPFM mixed with 30% methanol in the first season.

Table 4. Effect of spraying with *Methylobacterium*, methanol, or a combination of *Methylobacterium* on chlorophyll, foliage dry matter, and carbohydrate content of two strawberry cultivars during the 2017/2018 and 2018/ 2019 seasons

Characters	Chlorophyll reading (SPAD)			Foliage dry matter (%)			Carbohydrate (mg/g dry weight)		
Cultivars / Treatments	Florida	Festival	Mean	Florida	Festival	Mean	Florida	Festival	Mean
2017-2018 Season									
Control	46.66 i	48.67 h	**47.66 D**	20.72 f	18.91 g	**19.81 D**	4.670 f	4.787 e	**4.728 D**
10% methanol	49.77 g	51.57 cde	**50.67 C**	24.80 c	22.89 e	**23.84 BC**	5.653 b	5.557 c	**5.605 B**
30% methanol	50.70 f	50.57 f	**50.63 C**	23.92 d	22.54 e	**23.23 C**	5.446 d	5.542 c	**5.494 C**
PPFM + 10% methanol	51.93 bcd	52.22 b	**52.08 AB**	25.79 b	25.76 b	**25.78 A**	5.694 b	5.935 a	**5.815 A**
PPFM + 30% methanol	51.33 de	51.07 ef	**51.20 BC**	25.10 c	23.90 d	**24.50 B**	5.652 b	5.559 c	**5.606 B**
PPFM	53.00 a	52.13 bc	**52.57 A**	26.45 a	24.26 d	**25.35 A**	5.672 b	5.879 a	**5.777 A**
Mean	**50.57 A**	**51.04 A**		**24.46 A**	**23.04 A**		**5.464 A**	**5.544 A**	
2018-2019 Season									
Control	50.80 g	51.10 g	**50.95 E**	21.79 f	21.49 f	**21.64 C**	4.379 g	4.583 F	**4.481 D**
10% methanol	51.13 g	53.20 cd	**52.17 CD**	26.30 c	26.04 cd	**26.17 B**	5.603 d	5.466 e	**5.534 C**
30% methanol	52.37 e	51.67 f	**52.02 D**	25.46 de	25.94 cd	**25.70 B**	5.823 c	5.589 d	**5.706 B**
PPFM + 10% methanol	53.17 cd	53.50 c	**53.33 B**	27.70 a	27.30 ab	**27.50 A**	6.452 b	5.831 c	**6.142 A**
PPFM + 30% methanol	52.93 d	52.80 de	**52.87 BC**	26.66 bc	24.83 e	**25.75 B**	6.516 b	5.857 c	**6.187 A**
PPFM	55.87 a	54.57 b	**55.22 A**	27.91 a	27.43 ab	**27.67 A**	5.619 d	6.746 a	**6.183 A**
Mean	**52.71 A**	**52.81 A**		**25.97 A**	**25.50 A**		**5.73 A**	**5.68 A**	

Values within columns or rows followed by the same capital or small letter do not significantly differ from each other according to Duncan's multiple range test at 5% significance.

Table 5. Effect of spraying with *Methylobacterium*, methanol, or a combination of *Methylobacterium* on the fruit firmness, TSS, and acidity in two strawberry cultivars during the 2017/2018 and 2018/ 2019 seasons

Characters	Firmness (kg/cm)			TSS (%)			Acidity (mg/100g f.w)		
Cultivars / Treatments	Florida	Festival	Mean	Florida	Festival	Mean	Florida	Festival	Mean
2017-2018 Season									
Control	228.3 f	260 e	**244.2 C**	9.200 f	8.733 g	**8.967 C**	0.8937 b	0.8853 b	**0.8895 A**
10% methanol	281.7 cd	345 ab	**313.3 A**	10.80 c	10.27 d	**10.53 B**	0.8237 c	0.9113 ab	**0.8675 A**
30% methanol	275 de	288.3 cd	**281.7 B**	11.60 a	9.800 e	**10.70 B**	0.93 a	0.8157 cd	**0.8728 A**
PPFM + 10% methanol	328.3 b	293.3 cd	**310.8 AB**	10.60 c	10.00 de	**10.30 B**	0.8157 cd	0.7950 d	**0.8053 B**
PPFM + 30% methanol	280.0 d	343.3 ab	**311.7 AB**	11.67 a	11.27 b	**11.47 A**	0.6347 f	0.9320 a	**0.7833 B**
PPFM	300.0 c	361.7 a	**330.8 A**	11.60 a	11.60 a	**11.60 A**	0.6670 e	0.6457 ef	**0.6563 C**
Mean	**282.2 A**	**315.3 A**		**10.91 A**	**10.28 A**		**0.7941 A**	**0.8308 A**	
2018-2019 Season									
Control	253.3 f	271.7 e	**262.5 C**	9.867 f	9.867 f	**9.867 D**	0.9667 b	1.021 a	**0.9937 A**
10% methanol	286.7 d	368.3 a	**327.5 AB**	11.13 de	11.00 de	**11.07 C**	0.9397 bc	0.9343 c	**0.9370 B**
30% methanol	320.0 bc	315.0 bc	**317.5 B**	12.20 a	11.33 cd	**11.77 AB**	0.8747 d	0.8450 e	**0.8598 C**
PPFM + 10% methanol	318.3 bc	306.7 c	**312.5 B**	11.73 b	10.87 e	**11.30 BC**	0.7667 f	0.7563 fg	**0.7615 D**
PPFM + 30% methanol	310.0 c	370.0 a	**345.0 A**	12.30 a	11.73 b	**12.02 A**	0.6840 h	0.8293 e	**0.7567 D**
PPFM	324.0 b	380.0 a	**347.0 A**	12.27 a	11.60 bc	**11.93 A**	0.7280 g	0.7653 f	**0.7467 D**
Mean	**302.1 A**	**335.3 A**		**11.58 A**	**11.07 A**		**0.827 A**	**0.859 A**	

Values within columns or rows followed by the same capital or small letter do not significantly differ from each other according to Duncan's multiple range test at 5% significance.

3.5.4 Anthocyanins and ascorbic acid

Festival fruits had higher anthocyanin and ascorbic acid content than Florida fruits in both seasons (**Table 6**), consistent with the findings of El Badawy et al (2017). Moreover, PPFM spraying resulted in the highest anthocyanins and ascorbic acid values in both seasons. Ramadan and Omran (2005) reported that the application of methanol improved the total anthocyanin content in berry skins. Cruz-Rus et al (2011) attributed increased ascorbic acid content to increased synthesis of the six-carbon sugar derivative required for ascorbic acid biosynthesis. Furthermore, Festival plants treated with PPFM had the highest anthocyanin content in both seasons, followed by treatment with PPFM combined with 10% methanol. This finding was in accordance with the results of Rahman et al (2018) and Valizadeh-Kamran et al (2019). Further, the greatest effects on ascorbic acid content in Festival cv. were obtained by spraying with PPFM, a finding that may be attributable to the ability of PPFM to produce factors such as auxin, cytokinin, and vitamin B12 which may increase the biosynthesis of ascorbic acid (Koenig et al 2002).

Table 6. Effect of spraying with *Methylobacterium*, methanol, or a combination of *Methylobacterium* on the fruit anthocyanin, ascorbic acid, and potassium content of two strawberry cultivars during the 2017/2018 and 2018/2019 seasons

Characters	Total anthocyanins mg/100 g (f. w)			Ascorbic acid mg/100 g (f. w)			Potassium content (%)		
Cultivars / Treatments	Florida	Festival	Mean	Florida	Festival	Mean	Florida	Festival	Mean
2017-2018 Season									
Control	65.32 i	90.93 h	**78.12 D**	39.69 h	53.61 d	**46.65 D**	1.741 e	1.384 g	**1.563 C**
10% methanol	92.19 h	127.5 c	**109.8 C**	44.73 g	60.97 b	**52.85 C**	1.861 d	1.500 f	**1.680 B**
30% methanol	106.7 e	123.6 d	**115.2 B**	47.06 f	60.86 b	**53.96 C**	1.881 d	1.560 f	**1.721 B**
PPFM + 10% methanol	98.88 g	129.9 b	**114.4 B**	52.02 e	63.21 a	**57.61 B**	2.058 b	1.759 e	**1.908 A**
PPFM + 30% methanol	98.52 g	129.0 b	**113.7 B**	52.76 de	63.07 a	**57.92 AB**	1.954 c	1.530 f	**1.742 B**
PPFM	103.2 f	133.5 a	**118.4 A**	55.11 c	63.88 a	**59.49 A**	2.199 a	1.760 e	**1.979 A**
Mean	**94.14 B**	**122.4 A**		**48.56 B**	**60.93 A**		**1.949 A**	**1.582 A**	
2018-2019 Season									
Control	73.55 l	94.29 k	**83.92 E**	42.07 i	56.01 d	**49.04 D**	1.828 h	1.424 i	**1.626 D**
10% methanol	98.73 j	129.7 e	**114.2 D**	47.48 h	61.92 c	**54.70 C**	1.948 fg	1.973 f	**1.961 C**
30% methanol	102.3 i	139.6 c	**120.9 C**	49.19 g	62.47 c	**55.83 C**	2.167 e	1.910 g	**2.039 C**
PPFM + 10% methanol	109.8 h	144.2 a	**127.0 A**	53.92 e	64.85 a	**59.38 AB**	2.450 b	2.405 b	**2.427 A**
PPFM + 30% methanol	114.8 f	134.7 d	**124.8 B**	52.72 f	63.69 b	**58.20 B**	2.242 d	2.178 e	**2.210 B**
PPFM	111.7 g	141.3 b	**126.5 A**	55.80 d	64.67 a	**60.24 A**	2.507 a	2.295 c	**2.401 A**
Mean	**101.8 B**	**130.6 A**		**50.20 B**	**62.27 A**		**2.190 A**	**2.031 A**	

Values within columns or rows followed by the same capital or small letter do not significantly differ from each other according to Duncan's multiple range test at 5% significance.

3.5.5 Leaf potassium content

No significant differences in the potassium content of leaves were observed between the two strawberry cultivars, with PPFM greatly improving potassium content in both seasons (**Table 6**). Spraying with PPFM or PPFM combined with 10% methanol greatly increased leaf potassium content in both seasons. *Methylobacterium* sp. is a biostimulator and biofertilizer that directly influences plant growth by supplying nutrients to the plant host (Kwak et al 2014). The greatest effects on potassium content were observed in Florida plants treated with PPFM in both seasons.

3.6 SEM studies

SEM images of leaves from untreated strawberry plants and plants sprayed with PPFM alone or in combination with methanol are shown in **Fig 2**. PPFM was observed at stomata in the presence of methanol (**Fig 2B**). Strawberry leaves became hypersensitive after treatment with methanol (**Fig 2B and C**). When PPFM was combined with methanol, the harmful effects of methanol on leaf tissues were reduced (**Fig 2D and E**). Further, the use of PPFM alone maintained the regular structure of strawberry epidermal cells and had a beneficial effect on most of the evaluated characteristics (**Fig 2F**).

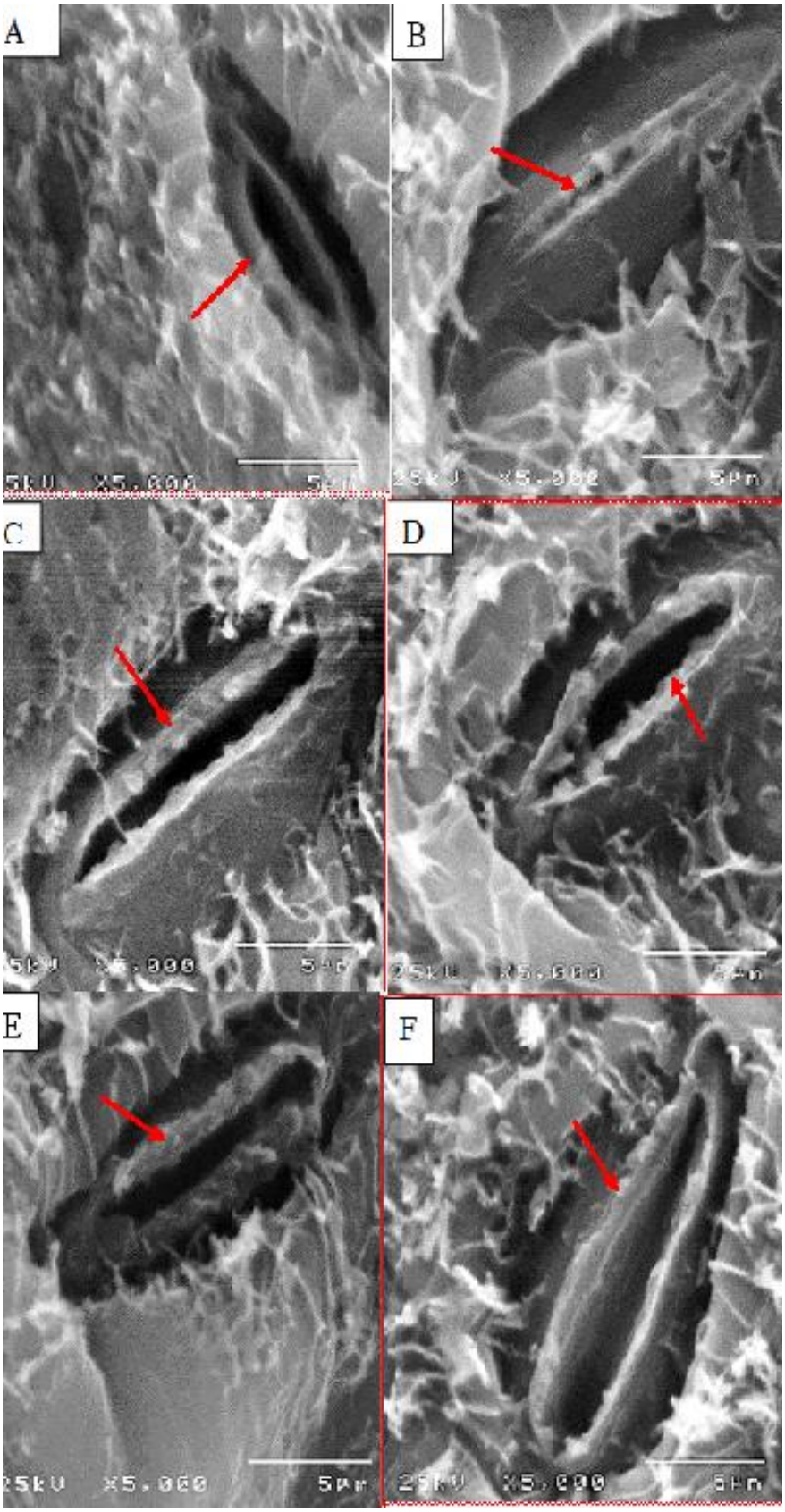

Fig 2. SEM photographs demonstrating epiphytic PPFM at the stomata of Strawberry plant for all treatments. A, distilled water; B, 10% methanol; C, 30% methanol; D, PPFM + 10% methanol; E, PPFM + 30% methanol; F, PPFM.

4 Conclusion

The results of the present study demonstrate that Florida cv. has a higher fresh weight and earlier yield than cv. Florida. Further, Festival cv. had a higher total yield per plant and per feddan. The anthocyanin and ascorbic acid content was higher in Florida plants in both seasons. PPFM spraying had the greatest effects on fresh weight, total yield per plant, total yield per feddan, anthocyanin content, ascorbic acid content and chlorophyll readings (SPAD). However, leaf area, dry matter percentage, early yield, fruit firmness, leaf potassium content, and carbohydrate content were highest in plants treated with either PPFM or PPFM combined with 10% methanol. PPFM treatment of Festival cv. produced superior fruit quality characteristics and the highest total yield.

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