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Research Report

Mitigating the adverse effects of deficit fertigation on strawberry yield, quality and phytochemical compounds by salicylic acid and putrescine treatments

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Abstract.
BACKGROUND: For global water shortage concerns and high cost of mineral nutrients it is necessary to decrease the amount of nutrient solutions in greenhouse production systems. Deficit fertigation may negatively affect the crop productivity and phytohormones can mitigate the adverse effects of stresses.
OBJECTIVE: We studied the effects of deficit fertigation in combination with salicylic acid (SA) and putrescine (PUT) on strawberry fruit yield and quality.
METHODS: Strawberry plants were fertilized with a complete nutrient solution of 220 (control), 180 (mild deficit fertigation) and/or 140 mL/D (severe deficit fertigation), and treated with PUT (at 0 and 2 mM) and/or SA (at 0 and 2 mM) and the combinations of these treatments during growth stages. Fruit growth, quality parameters, yield and phytochemical compounds were evaluated at harvest.
RESULTS: Mild deficit fertigation (MDF) (140 mL/D) significantly enhanced the yield and quality of the fruit, and both PUT and SA, enhanced the positive effects of MDF on crop productivity. SA and PUT decreased the negative effects of DF on crop yield and fruit growth.
CONCLUSIONS: The results of this study indicate that it is possible to substantially enhance the quality and productivity of strawberries with a MDF regime, PUT and SA treatments.

Keywords: Strawberry fruit quality, deficit fertigation, phenolics, putrescine, salicylic acid

1. Introduction

Strawberry (*Fragaria*×*ananassa*) is a popular fruit known for the special flavor and taste, essential nutrients, phytochemicals and health-promoting properties. For being rich in different phenolics, vitamins and powerful antioxidants the strawberry fruit juice plays important roles in improving human health and prevention of different oxidative related diseases including different cancers [1]. In Iran, most of the strawberries are produced in greenhouses under hydroponic conditions with a relatively high cost. Decreasing the need for water and nutrient

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solutions, called as deficit fertigation, is a method for decreasing the production costs [2, 3]. Furthermore, the water shortage is a main problem in most regions and irrigation with water below the optimum crop requirements is a strategy for saving water. On the other hand, the environmental impact of the release of potentially eutrophying elements is a global problem and it is necessary to decrease the use of these nutrients in irrigation water of the crops. Some reports indicate that a decrease in nutrient level may has no significant effects on crop yield and quality [4]. Recently researchers have introduced the deficit fertigation method as an efficient strategy to decrease both the water and nutrients use [2–4]. In this method the plants are subjected to a certain level of water stress and lower nutrient concentrations either during a particular period or throughout the whole growing season [2, 3]. Since decreased irrigation levels may act as a biotic stress, then in order to avoid the possible negative effects of the stress, it is necessary to enhance the stress resistance mechanisms of the plant under decreased water conditions [5]. In addition, decreased nutrient levels may result in a decline in the crop yield and quality, and it is necessary to enhance the plant productivity and nutrient uptake capacity by proper management tools including the phytohormones and plant growth regulators (PGRs).

Recently the use of phytohormones and PGRs has been considered for managing plant responses to different environmental conditions and for enhancing the crop yield and quality (6–8). These phytohormones have been shown to be involved in plant resistance responses against environmental stresses as well as water shortage and nutrient deficiency conditions [6–9]. Some of these phytohormones such as polyamines and salicylates are able to enhance plant photosynthesis reactions under water stress conditions by increasing rubisco enzyme activity, CO_2 enrichment rate, protein synthesis, osmolyte production, water and nutrient absorption rate, cell enlargement and division capability and antioxidant capacity, and decreasing ethylene production and action, oxidative stress and evapotranspiration rate [9–17]. Furthermore, the role of these phytohormones in enhancing crop overall and nutritional quality including color, size, dimensions, antioxidant capacity, taste and flavor, phenolics and vitamins under normal growth conditions has been demonstrated [9, 12, 18].

Salicylic acid (SA) has been shown to enhance strawberry fruit antioxidant activity and quality parameters and the plant stress resistance mechanisms under both normal and water stress conditions [19, 20]. It has been reported to increase total leaf area, leaf and shoot dry matter, leaf number, proline and soluble carbohydrate content, catalase and peroxidase activity and crop yield of strawberries under deficit irrigation conditions [20].

Putrescine is a famous member of polyamines, which are recognized as a group of phytohormones with anti-stress activities. Putrescine, like other polyamines, is an antioxidant molecule and has been shown to activate different antioxidant systems of the plants under stress conditions. The role of putrescine in mitigating the adverse effects of salt and water stress by modulating water and nutrient ions uptake, and enhancing antioxidant capacity and water use efficiency in strawberries and some other plants has been demonstrated [15, 21, 22]. Both salicylates and polyamines have been shown to prevent chlorophyll degradation and protect photosynthesis apparatus under stress conditions resulting in the production of assimilates. By activating the secondary metabolism these phytohormones may enhance the phytochemical contents of the fruit both under normal and stressful conditions [9, 16, 19, 20, 21, 23].

It seems that the combination of SA and putrescine, as safe and environmental friendly stress mediating phytochemicals, may have more positive effects in improving tolerance mechanisms against water stress and decreased nutrient levels. Since there is no report regarding the combination effects of these two phytochemicals on strawberry yield and fruit quality parameters under deficit fertigation conditions, then this study was conducted to determine if SA and putrescine are able to mitigate the probable adverse effects of deficit fertigation on strawberry fruit quality and yield or not.

2. Materials and methods

2.1. Plant material and experimental design

Strawberry transplants (*Fragaria* × *ananassa* Duch. cv. 'Sabrina'), were obtained from a commercial production center and planted in Research Greenhouse of Horticulture Department (Urmia University, Urmia, Iran) in

Table 1
Amounts of nutrients used for growing strawberries in a hydroponic system according to Lynette (2006)

Growth stage	Nutrients (mg/L)													
	N	P	K	Mg	Ca	S	Fe	Mn	Zn	B	Cu	Mo	EC	pH
Vegetative growth	207	65	184	58	221	77	6.5	2.6	0.25	0.7	0.07	0.05	2	5.8
Fruiting	182	82	301	58	148	77	6.5	2.6	0.25	0.7	0.07	0.05	2	5.8

a hydroponic system on October 24, 2018. The culture medium was a mixture of coco-pit and perlite (1:1), and the greenhouse condition was set to 22°C/15°C, day/night, and 60–80% RH. Artificial light was provided during the cloudy days.

The experiment was designed as a completely randomized design with 2 (SA concentrations) × 2 (PUT concentrations) × 3 (nutrient solution volume) = 12 factors, and in 4 replicates (each plant was considered as one replication). Then we had 48 plants for the experiment.

In order to treatment of plants with different fertigation levels, the plants were daily irrigated with 220, 180 and/or 140 mL nutrient solutions, where 220 mL/day was considered as control, 180 mL/day as mild deficit fertigation (MDF) and 140 mL as severe deficit fertigation (SDF) regimes. The nutrient solutions were prepared according to the recommendations of Lynette [24] (Table 1). For salicylic acid (SA) and putrescine (PUT) treatments the plants were treated with 0 and/or 2 mM of each phytohormone every 20 days. The first phytohormone treatment was performed 20 days after planting. For combination treatments the plants were first sprayed with SA and then were treated with PUT. In a period of 30 days from the ripening of the first fruit for each plant the fruit were harvested and subjected to different evaluations. The length of experiment from the planting until the final harvest was 120 days. The fruits from the control and treated plants were harvested at commercial ripening, when more than 75 % of fruit was red colored, in the morning and immediately transferred to plant physiology laboratory of Urmia University.

2.2. Total soluble solids (TSS) content, total acidity (TA), palatability and pH determination

In order to prepare the fruit juice for determination of TSS, TA and pH, 5 fresh fruit of each replicate were halved and homogenized in a blender and the homogenate was filtered through the cheesecloth. TSS in the extracted juice was measured using a digital refractometer and the results were expressed as %. TA was determined by diluting each 5 mL aliquot of strawberry juice in 95 mL of distilled water and then titrated to pH = 8.2 using NaOH (4 g/L) [25]. According to the method described by Voca et al. [26], the ratio of soluble solids to total acidity was considered as palatability, sweetness, index. pH of the fruit juice was measured using a digital pH-Meter (CG824).

2.3. Total antioxidant activity (TAA) and total phenolics determination

For total antioxidant and total phenolics evaluations the extract was prepared according to the method described by Ariza et al. [27] with a slight modification. 0.5 mL of strawberry juice was mixed with 5 mL methanol:water mixture (80:20, v/v). The resulting homogenates were stirred at room temperature in the dark for 2 h and then centrifuged at 3000 rpm for 15 min, the supernatant was filtered and the hydromethanolic extracts were stored at –20°C until analysis. Total antioxidants were determined using DPPH radical scavenging assay [28]. For this purpose, 100 μL extract of samples was added with 3 mL of 4 % DPPH solution (0.004 g DPPH in 100 mL methanol), and the mixture was kept at room temperature for 20 min in the dark. The absorbance changes were read with a spectrophotometer at 517 nm. The radical scavenging activity was calculated by the following equation:

$$\text{DPPH}\,(\%\text{inhinition}) = \left[(\text{Abs}_0 - \text{Abs}_1)/\text{Abs}_0\right] \times 100 \quad (1)$$

where Abs_0 is absorbance value of blank sample, and Abs_1 is absorbance reading of sample.

2.4. Total phenolics content (TPC)

Total phenolics were determined according to Folin–Ciocalteu method [29]. Briefly, 100 μL of hydromethanolic extract was mixed with 500 μL of Folin–Ciocalteu reagent (10%) and incubated for 3 min then 400 μL of CH_3CO_2Na solution (7 M) was added. After 2 h of incubation at room temperature in the dark, the UV–Vis spectrophotometer was used to measure the absorbance at 760 nm. Gallic acid was used as standard and the results were expressed in milligrams of gallic acid equivalents (GAE) per 100 g fresh weight (mg GAE/100 g FW).

2.5. Ascorbic acid (AsA) content

AsA content was measured according to the method described by [30]. The AsA content content was expressed as mg ascorbic acid/kg FW. Fresh fruit tissue (5 g) was homogenized and mixed with 100 mL metaphosphoric acid (6%) containing 2 M acetic acid to measure AsA content. The mixture was centrifuged at 18,000 g for 15 min at 4°C. Then the supernatant was filtered and 1 mL aliquot of the supernatant was mixed with 0.05 mL 2, 6-dichlorophenolindophenol (0.2%), and the solution was incubated at room temperature for 1 h. Finally, 1 mL thiourea (2%) in metaphosphoric acid (5%) and 0.5 mL of dinitrophenyl hydrazine (2%) in 4.5 mL/L sulfuric acid were added to the solution and incubated at 60°C for 3 h. The reaction was stopped by placing the tubes in ice bath and slowly adding 2.5 mL of ice cold sulfuric acid (2%). Spectrophotometric measurements of AsA content were taken at 520 nm. The results were compared to a calibration curve derived from data from ascorbic acid (100 $\mu g\ mL^{-1}$) in 0.5% oxalic acid.

2.6. Total Anthocyanin content (TAC) determination

Frozen fruit (10 g) were ground with mortar and pestle in the presence of liquid nitrogen. Approximately 0.3 g of the resultant powder was poured into 3 mL of HCl–methanol 1 % (v/v) and kept at 0°C for 10 min. The slurry was centrifuged at 1500 g at 4°C for 10 min, the supernatant was collected and its absorbance at 515 nm was measured. The amount of anthocyanins was expressed as micromoles of pelargonidine-3-glucoside per Kg fruit, using Emolar = 3.6×10^6 M/L m) [31].

2.7. Fruit firmness

Strawberry fruit firmness, all fruit just after harvest, was measured using a digital penetrometer (Texture analyzer) fitted with a P_5 probe with the cylindrical flat bottom and the diameter of 5 mm. The fruit was penetrated to a depth of 7 mm in the fruit shoulder zone with test speed of 0.5 mm/s. The results are reported as the peak force in newton (N) [32].

2.8. Length, diameter and shape of the fruits

The fruit length and diameter were measured using a digital caliper. Fruit shape was evaluated using a hedonic nine-point scale, anchored in the extremes “dislike very much” (score 1) and “like very much” (score 5) [33].

2.9. Crop yield

The fruit from all plants were harvested within 30 days starting from the ripening of the fruit from the first flower. The sum of the fruit weight harvested in harvest period was considered as the yield of each plant.

2.10. Statistical analysis

All statistical analyses were performed according to the general linear model procedure of statistical analysis system (SAS, version 9.2) and mean separations were performed by Duncan's multiple range test. Differences at $p \leq 0.01$ and $p \leq 0.05$ were considered as significant.

3. Results

3.1. Fruit TSS, TA content and palatability

Both SA and fertigation regime significantly ($p \leq 0.5$ and $p \leq 0.1$, respectively) affected fruit TSS content (Table 2). With decrease in the amount of nutrient solution TSS content was increased (Table 3). Both SA and Put enhanced fruit TSS content under control and deficit fertigation conditions but the differences were not significant (Tables 2, and 3). The lowest TSS content was recorded in control fruit, grown under normal fertigation regime and received no phytohormone. Fruit TA was significantly affected by all treatments (Table 2). Deficit fertigation decreased the fruit TA and the highest and lowest TA was recorded in control fruit and fruit grown under SDF, respectively (Table 3). The TA content of control fruit was increased in response to SA and PUT treatments, but the difference was significant in combination treatments. Fruit palatability index was significantly affected by SA and DF treatments ($p \leq 0.1$). The lowest palatability was recorded in control fruit receiving no deficit fertigation or phytohormone treatment, and the highest value was recorded in fruit received SDF showing no significant difference with the fruit received SA and/or PUT under MDF (Table 3). Both SA and PUT treatments significantly enhanced the palatability and acceptance index of the fruit grown under MDF condition.

3.2. pH

pH of the fruit juice was affected by DF (Table 2), and the interaction effects of SA and PUT with DF was also significant ($p \leq 0.01$) (Table 3). The lowest pH was recorded in control fruit received 220 mL/d nutrient solution and did not treated with PUT or SA, while the highest value belonged to SDF with no PUT or SA treatments. These data indicate that SA and PUT are able to prevent from the dramatic increase in fruit pH in response to SDF.

3.3. TAA

Fruit juice antioxidant activity was significantly affected by the treatments and fertigation regime ($p \leq 0.1$) (Table 2). Although, all treatments enhanced fruit TAA, but the highest TAA was recorded in fruit treated with SDF (Table 3). The lowest TAA was recorded in control fruit receiving no phytohormone treatment and SA and PUT significantly enhanced fruit TAA under normal fertigation and MDF regimes.

Table 2
ANOVA for the effects of fertigation (F), salicylic acid (SA) and putrescine (PUT) on different quality parameters, phytochemical compounds and yield of strawberry fruit

Source	DF	Mean Squares											
		TSS	TA	Taste	pH	TAA	Total phenolics	AsA	TAC	Firmness	Fruit length	Fruit diameter	Fruit shape	Yield
SA	1	2.066*	0.024*	4.46**	0.0003^{ns}	715.798**	1027.91**	0.001^{ns}	41.103**	0.0001^{ns}	6.63^{ns}	0.21^{ns}	0.076^{ns}	2908.211**
PUT	1	0.177^{ns}	0.05**	0.824^{ns}	0.0008^{ns}	65.894**	9762.57**	0.002^{ns}	234.76**	0.044**	0.388^{ns}	0.913^{ns}	0.083^{ns}	3003.949**
F	2	9.350**	0.117**	27.24**	0.027**	965.301**	88235.95**	0.004**	1471.1**	0.067**	9.675*	4.517**	2.402**	1616.213**
SA × PUT	1	0.116^{ns}	0.005^{ns}	0.178^{ns}	0.001^{ns}	103.488**	133.006^{ns}	0.004**	78.19*	0.088**	63.48**	8.217**	2.576**	562.528**
SA × F	2	0.139^{ns}	0.032**	0.730^{ns}	0.006**	308.990**	5175.275**	0.001^{ns}	305.84**	0.048**	16.145**	4.22**	0.276*	10.344^{ns}
PUT × F	2	1.378**	0.037**	3.665**	0.006**	346.054**	860.048**	0.001^{ns}	436.85**	0.022**	13.803**	2.912*	0.347*	25.249^{ns}
SA × PUT × F	2	1.786**	0.022**	4.437**	0.004^{ns}	153.028**	21163.64**	0.002^{ns}	0.30^{ns}	0.002^{ns}	8.811*	0.191^{ns}	0.238^{ns}	25.250^{ns}
Error	36	0.233	0.003	0.413	0.0008	1.524	61.922	0.0005	0.698	0.0035	2.036	0.578	0.073	20.41
CV (%)		5.309	5.618	7.581	0.798	1.475	2.067	5.806	3.782	4.745	3.668	2.611	6.530	6.915

^{ns}, * and ** means NO significant and significant at $p \leq 0.05$ and $p \leq 0.01$ levels, respectively.

Table 3
Changes in strawberry fruit soluble solids content (TSS), total acids (TA), palatability (taste), total antioxidant activity (TAA), total phenolics content (TPC) and ascorbic acid (AsA) in response to different fertigation regimes, and foliar spray with PUT and SA during growth stages

Treatments			Quality parameters*						
SA (mM)	PUT (mM)	Fertigation (mL/d)	TSS (Brix°)	TA (g/100 mL)	Taste (TSS/TA)	pH	TAA (% DPPH inhibition)	TPC (mg/100 g)	AsA (mg/100 mL)
0	0	220	8^{c}	1.38^{a}	5.81^{c}	3.55^{c}	55.54^{h}	263.66^{i}	0.355^{c}
0	0	180	8.31^{bc}	1.04^{cde}	7.43^{bc}	3.66^{c}	81.68^{f}	370.35^{e}	0.388^{c}
0	0	140	10.38^{a}	0.95^{e}	10.71^{a}	3.72^{a}	94.25^{a}	531.44^{a}	0.408^{bc}
0	2	220	8.66^{bc}	1.11^{ab}	7.79^{ab}c	3.6^{c}	78.31^{g}	322.55^{g}	0.381^{c}
0	2	180	8.92^{bc}	1.06^{cde}	8.42^{ab}	3.65^{b}	81.08^{f}	348.11^{f}	0.412^{bc}
0	2	140	9.04^{bc}	1.02^{cde}	8.88^{ab}	3.67^{b}	87.92^{cd}	419.22^{c}	0.457^{a}
2	0	220	8.94^{bc}	1.14^{ab}	7.83^{bc}	3.59^{c}	82.17^{de}	375.88^{e}	0.374^{c}
2	0	180	9.07^{bc}	1.07^{bc}	8.46^{abc}	3.64^{b}	90^{bc}	399.22^{d}	0.394^{bc}
2	0	140	10.22^{b}	1.04^{cde}	9.84^{ab}	3.66^{b}	91.28^{b}	428.11^{c}	0.432^{b}
2	2	220	8.15^{bc}	1.07^{bc}	7.6^{bc}	3.64^{b}	85.44^{cd}	293.66^{h}	0.368^{c}
2	2	180	9.33^{b}	1.04^{cde}	8.97^{ab}	3.66^{b}	86.63^{de}	350.33^{f}	0.386^{c}
2	2	140	10.09^{b}	1.01^{de}	9.99^{ab}	3.65^{b}	89.6^{bc}	463.66^{b}	0.426^{b}

*Different letters for the data of each group (each parameter) indicates that the difference is significant at $p \leq 0.01$ according to Duncan's multiple range test.

3.4. TPC

All treatments affected fruit TPC ($p \leq 0.1$) (Table 2). Control fruit showed the lowest TPC, while the highest value belonged to fruit received the SFD (Table 3). The response of the fruits in terms of total phenolics was different under different fertigation regimes. Under MDF condition the highest TPC was seen in fruit treated with SA, while PUT enhanced these phytochemicals in control fruit.

3.5. AsA content

Fruit AsA content was significantly enhanced by deficit fertigation and the fruit received SDF showed the highest ascorbic acid content ($p \leq 0.01$) (Table 2). Both SA and PUT enhanced the vitamin C content of fruit in all fertigation treatments. The highest ASA content was recorded in fruit treated with PUT + SDF (Table 3).

3.6. TAC

Total anthocyanins, as a group of powerful antioxidants, were significantly affected by DF and the treatments ($p \leq 0.01$) (Table 2, Table 4). When the plants received no phytohormone treatment the highest TAC was seen in fruit received SDF, but PUT and SA treatments enhanced the positive effects of MDF in enhancing fruit TAC. Furthermore, the lowest amount was recorded in control fruit received no DF, and the phytohormones enhanced AsA under normal fertigation (Table 4).

3.7. Fruit firmness

The firmness index of fruits was significantly affected by PUT and DF treatments ($p \leq 0.01$) (Table 2). SA as single treatment decreased the fruit firmness, but positively affected it when combined with PUT or DF

Table 4
Changes in strawberry fruit total anthocyanin content (TAC), fruit firmness, length, diameter, shape and yield in response to different fertigation regimes, and foliar spray with PUT and SA during growth stages

Treatments			Quality parameters*					
SA mM	PUT mM	Fertigation (mL/d)	TAC (μM/kg)	Tissue firmness (N)	Fruit length (mm)	Fruit diameter (mm)	Fruit shape (score)	Crop yield (g/plant)
0	0	220	90.77^{c}	1.247^{bc}	35.09^{c}	27.22^{c}	3.12^{c}	191.278^{c}
0	0	180	129.42^{b}	1.361^{b}	40.12^{a}	29.64^{ab}	4.35^{ab}	278.013^{ab}
0	0	140	184.32^{a}	1.227^{bc}	39.43^{ab}	29.06^{ab}	4.30^{ab}	230.088^{b}
0	2	220	120.92^{bc}	1.208^{c}	39.33^{ab}	28.77^{b}	4.1^{b}	237.318^{b}
0	2	180	158.9^{ab}	1.249^{bc}	41.38^{a}	29.92^{a}	4.7^{a}	314.506^{a}
0	2	140	168.33^{ab}	1.302^{b}	37.88^{b}	30.54^{a}	4.45^{ab}	248.423^{b}
2	0	220	118.96^{bc}	1.117^{d}	38.4^{b}	28.65^{b}	3.9^{b}	235.64^{b}
2	0	180	141.13^{b}	1.253^{bc}	40.33^{a}	29.75^{a}	4.52^{a}	298.89^{a}
2	0	140	162.45^{ab}	1.274^{bc}	38.07^{b}	29.42^{ab}	4.32^{ab}	239.705^{b}
2	2	220	141.65^{ab}	1.239^{bc}	38.63^{b}	29.24^{ab}	3.75^{b}	234.596^{b}
2	2	180	165.72^{ab}	1.260^{bc}	36.89^{c}	28.12^{b}	3.92^{b}	220.448^{bc}
2	2	140	145.18^{b}	1.507^{a}	38.88^{b}	28.99^{b}	4.1^{b}	169.57^{d}

*Different letters for the data of each group (each parameter) indicates that the difference is significant at $p \leq 0.01$ according to Duncan's multiple range test.

(Table 2). According to Table 4 fruit received no DF and treated with SA had the lowest firmness with no significant difference with control fruit, while the highest firmness belongs to fruit received SDF + SA + PUT (Table 4).

3.8. Fruit length, diameter and shape

Fruit growth rate, as indicated by dimensions including the length and diameter, was affected by fertigation treatment (Table 2). The highest fruit length was recorded in fruit received MDF. Although SA and PUT, as single treatments, had no significant effect on fruit length, but enhanced this parameter under DF regimes (Table 4). The lowest fruit length belonged to the control plants which received no DF or phytohormone treatment.

Fruit diameter was decreased in response to 220 mL/d fertigation regime (Table 4). The lowest diameter was recorded in control fruit, while the fruit received mild or severe deficit fertigation with PUT had the highest diameter. In addition, SA had positive effects on this parameter under different fertigation treatments.

Fruit shape was significantly affected by fertigation treatment ($p \leq 0.05$) and the interaction effects of SA or PUT with DF was also significant (Table 2). With the significant changes in length and diameter of the fruits, the shape of fruits was affected by the treatments. According to Table 4, fruit received 180 mL/day fertigation + PUT had the highest shape score (Table 4). However, SA treatment significantly enhanced the fruit shape under MDF. Control fruit, received only 220 mL/d fertigation, had the lowest shape score. Although, SDF decreased the number of excellent fruits in terms of shape, but both SA and PUT, when applied as single treatments, significantly enhanced fruit shape under this fertigation regime.

3.9. Crop yield

Crop yield per plant was significantly affected by all treatments ($p \leq 0.01$) (Table 2). Furthermore, MDF significantly enhanced the crop yield, and the interaction effects of SA, PUT and DF was significant (Table 4). According to Table 4, treatment of plants with SA and/or PUT under MDF resulted in a higher yield performance.

The highest yield was recorded in plants received 180 mL/day fertigation regime + PUT with no significant difference with MDF and MDF + SA. The lowest crop yield was recorded in plants received SDF + PUT + SA, indicating the adverse effects of these combination on primary metabolism.

4. Discussion

Although, in this study, severe deficit fertigation enhanced all phytochemical compounds including antioxidants, phenolics and anthocyanins of the fruit, but highest overall quality was recorded in fruit received MDF. Furthermore, this fertigation regime showed the highest yield per plant. These findings indicate that with increase in fruit phytochemical compounds the yield and overall quality are decreased in response to SDF. Fertigation deficit treatment includes but decreased water and nutrient shortage and SDF may decrease both water and nutrient availability for the plants. The balance between phytochemicals, overall quality parameters and yield is very important for strawberry fruit productivity [3, 20, 25]. Fruit growth and development and the formation of different phytochemicals and quality parameters are the consequence of a series of complex processes including primary and secondary metabolisms [34]. Fruit formation and growth depends mostly on primary metabolism routes resulting in cell enlargement and division, while the formation of phytochemicals including different antioxidants, phenolics and anthocyanins depends on the activation of secondary metabolism routes [34–36]. The higher total acidity content in the fruits of plants received higher amount of nutrient solution in this study, may confirm this deduction, because organic acid accumulation is the consequence of primary metabolism [34]. The activation of each metabolism route in the plants and fruits is under the control of environmental factors, phytohormones, growth regulators, water and nutrient status [6, 7, 34–39]. Creating a balance between the two types of metabolic routes is critical for the production of high quality fruits with a high performance and crop yield. This is very important in fruits like strawberries which both the overall quality including the color, size and shape, and the phytochemical contents including total antioxidants, phenolics, taste and ascorbic acid content are strongly considered by the consumers [8, 29, 33, 40].

Our results indicate that higher amounts of water and nutrients may decrease strawberry fruit quality and yield. Some farmers believe that with supplying higher water and mineral nutrients the plant will produce much more fruit and they will earn higher incomes. While our data showed that a mild deficit fertigation may enhance both the quality and productivity of the strawberries. This is because, excess water decreases the yield by encouraging the vegetative growth of the plant, and the higher amounts of the nutrients also may decrease the plant photosynthesis efficiency and damage the normal cell metabolism by inducing mineral toxicity effects resulting in a decreased fruit growth, quality and yield [34, 41]. In addition, most of the conditions favorable for the growth processes are unfavorable conditions for the activation of secondary metabolism routes and formation of phytochemical compounds [3, 34, 41– 44]. Our results indicate that 220 mL/d nutrient solution not only decreases the yield but also has a strong negative effect on different overall quality parameters and different valuable phytochemicals. This will result in an increase in production costs, due to increased nutrient and water usage, and decreased crop yield and quality. In addition, both the yield and fruit quality were substantially decreased in response to severe deficit fertigation regime, 140 mL/d solution, indicating the negative effects of this stressful condition for the plants. In fact, under DF the plants receive both lower water resulting in a water stress, and inadequate mineral nutrients. It has been reported that deficit irrigation or partial root-zone drying deficit irrigation (PRD), may significantly reduce the yield in strawberries and severe water shortages may represent significant economic losses for farmers and are not recommended for improving strawberry water use efficiency [44]. Water stress results in stomatal closure leading to decreased photosynthesis and assimilate production rate resulting in subsequent reduction in fruit growth [34]. Accumulation of TA in plants under stressful conditions is decreased, because in response to stresses the acids are converted to soluble carbohydrates [35, 45– 47]. It seems that mild deficit fertigation, like deficit irrigation, acts as an activator of secondary metabolism resulting in the biosynthesis and accumulation of ascorbic acid, phenolics, soluble sugars and anthocyanins [3, 40]. Our

results show that 140 mL/day fertigation regime may disturb plant primary metabolism due to both water and nutrient shortage, resulting in decreased photosynthesis, fruit growth and yield. Previous reports indicate that in most strawberry cultivars over 30% of water shortage may lead to a significant loss in fruit yield, and the use of deficit irrigation strategies below this threshold is not recommended in strawberry production systems. Our finding is in agreement with these reports [44].

Both SA and PUT were able to enhance fruit phytochemical contents, quality parameters and yield under normal and mld fertigation treatment. While under SDF the combination of these phytohormones damaged the yield and quality parameters and enhanced phytochemical compounds. It has been demonstrated that some phytohormones such as polyamines, jasmonates, brassinosteroids, abscisic acid and salicylates are able to act as elicitors for the production of secondary metabolites [7, 17, 37, 38, 48]. SA and PUT are anti-stress phytohormones which are able to activate different stress resistance mechanisms in the plants and fruits, under normal and stressful conditions [9, 12, 23, 49]. The role of these compounds in enhancing the resistance mechanisms against water resistance and mineral shortage or toxicity has been reported in some plants [11, 37, 48, 50, 51]. SA and PUT are antioxidant compounds and have been reported to activate different antioxidant systems of the plants and fruits under normal and stress conditions [9, 23, 49, 53–55]. Oxidative stress, caused by different reactive oxygen species (ROS) and free radicals, is a main cause for the damage for plant cells and normal metabolism routes under different stress conditions, and the role of salicylates and polyamines is critical in detoxifying these dangerous molecules and preventing oxidative burst under both water stress and mineral toxicity conditions [12, 41, 52, 56, 48]. In this study, SA and PUT were able to enhance overall quality indices, yield, and antioxidant activity, phenolics and anthocyanin content of the fruit under normal and MDF. Phenolics and anthocyanins are powerful antioxidants and anti-stress compounds and play an important role in strawberry fruit quality and health promoting capacity [7, 48]. It seems that SA and PUT are able to enhance fruit different quality parameters, yield and phytochemicals by enhancing plant photosynthesis rate and secondary metabolism. These phytohormones are able to enhance water and nutrient absorbance rate and water use efficiency in plants resulting in increased photosynthesis rate under normal and stressful conditions [10, 13, 15, 16, 18, 21, 34, 48]. By modulating the pH of root cells and the activity of hairy roots these phytohormones are able to modulate the rate of water and mineral absorption under drought and salt stress conditions [13–16, 21, 48].

Strawberry fruit growth and development duration is substantially influenced by environmental and hormonal fluctuations [3, 39, 57]. It has been shown that stressful conditions, mainly water stress, is able to significantly decrease fruit growth duration and enhance early ripening, which results in the production of small fruits and decreased crop yield [45, 57, 58]. Recently the substantial role of abscisic acid (ABA) in strawberry fruit early ripening has been demonstrated, and it has proposed that this is the main phytohormone playing role in ripening process of this fruit [59]. Osmotic stress caused by water shortage results in accumulation of ABA which in turn inhibits root growth and extension [60]. According to our findings in this study, the fruits from the plants treated with SA showed a lowest firmness and higher anthocyanin contents, which may confirm the possible role of this phytohormone in strawberry fruit ripening. While, at the same time, SA enhanced fruit growth and crop yield, probably via prevention of ABA production, which the mechanisms may be more illustrated during the future molecular studies. Furthermore, the role of polyamines in prevention of ABA production and accumulation, and activation of SA production has been well demonstrated, explaining another possible mechanism of PUT in enhancing fruit growth rate [10, 37–38, 48]. Auxins, cytokinins and gibberellins are known as growth promoting phytohormones and the higher rate of fruit growth is an indicator of higher levels of these phytohormones in the plant and fruit [34, 39]. SA and PUT may be directly and indirectly involved in enhancing the yield, increasing the size and enhancing the shape of the fruits. Salicylates and polyamines have been shown to enhance the biosynthesis and stability of growth promoting phytohormones under normal and stress conditions, resulting in enhanced fruit growth under normal conditions and maintenance of growth potential under stressful conditions [38, 61–63]. Furthermore, polyamines have been reported to be directly involved in fruit growth processes via enhancing cell enlargement and division, and assimilate transport in plants [11, 23, 48, 51].

Interestingly, the combination of SA and PUT enhanced most of the measured parameters under normal conditions. Also, both SA and PUT, when applied as single treatments, mitigated the adverse effects of SDF and enhanced the yield parameters by activating different antioxidative and anti-stress mechanisms. While, the combination of these compounds under SDF decreased some quality parameters and yield performance. Nevertheless, because of the stomatal closure and declined photosynthesis rate, the substrate production rate is dramatically decreased in severe drought stress conditions. Therefore, more activation of a complex series of secondary routes in plant cells in response to combination of salicylic acid, putrescine and severe deficit fertigation treatments may result in consumption of the carbohydrate substrates for the production of resistance mechanisms resulting in the lack of substrates for being used in primary route [35, 63]. As seen in this study, this may result in a substantial increase in phenolic compounds and anthocyanins, as the products of secondary routes, and a significant decrease in fruit growth parameters and yield, as the result of decreased primary metabolism rate. On the other hand, combination of draught stress with a relatively higher concentrations of SA and PUT, which were used in this study, may result in the production of some toxic compounds such as higher levels of nitric oxide (NO) resulting in a more decrease in growth parameters. NO is an active nitrogen species that is generated in plant cells under stress conditions and in response to stress phytohormones, like polyamines and salicylates, and activates a series of resistance mechanisms including drought stress [9, 14, 17]. But over production of NO, as a free radical, in response to combination of SDF, PUT and SA is harmful and may damage the cells [34, 64], a subject which remains to be verified and more illustrated during future studies.

5. Conclusions

In this study, as a novel approach, the responses of strawberries to deficit fertigation, and salicylic acid and putrescine treatments were studied. Strawberry fruit yield, phytochemical content and quality parameters showed significant changes in response to different water and nutrient solution supply programs as well as the salicylic acid and putrescine treatments. Mild deficit fertigation enhanced all measured quality attributes, phytochemicals and yield parameters of the crop, and the treatments, alone and in combination, had positive effects on these responses. In addition, salicylic acid and putrescene, enhanced the fruit overall quality, antioxidants, phenolics, vitamin C, total soluble solids and growth rate under normal fertigation condition, indicating the roles of these compounds in activating the secondary metabolism. Furthermore, this may indicate the roles of these signaling compounds in decreasing the adverse effects of over irrigation and excess nutrition. Meanwhile, these phytohormones when applied alone had a positive effect on the measured parameters under severe deficit fertigation. These findings indicate that for the production of a high quality strawberry fruits with a higher yield, the fruit should be grown under mild deficit fertigation regimes, which results in a decreased production cost, decreased environment pollution and increased crop productivity, and SA or PUT may be used to enhance the crop productivity in a such system. Although a severe deficit fertigation decreases the yield and fruit overall quality but it is possible to decrease these negative effects with the use of salicylic acid or putrescine. Since in this study we examined a relatively higher levels of these phytochemicals, it seems that in future studies a combination of these phytohormones with a lower concentration may result in better responses under deficit fertigation regimes. More molecular studies with the use of salicylic acid and putrescine combinations is needed to illustrate the reasons for decreasing the strawberry fruit yield and overall quality parameters under severe deficit fertigation.

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Conflict of interest

The authors have no conflict of interest to report.

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