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Improved Water Use Efficiency and Yield of Drip-Irrigated Pepper under Full and Deficit Irrigation Conditions

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IN EGYPT, water scarcity is the main problem the agricultural sector faces. Salicylic acid (SA) as a plant growth regulator may help mitigate the adverse effects of deficit irrigation water on pepper plants. Sweet pepper (cv. S702 F1) plants subjected to deficit irrigation ($DI_{40\%}$= 60% of crop evapotranspiration, ETc) regularly during growth stages produced decreases in plant height, total chlorophyll, fruit number per plant, fruit length, fruit diameter, fruit volume, fruit yield, fruit carotenoids, and total dry matter comparing with the full irrigation (FI= 100% of ETc). However, water use efficiency (WUE), proline, free amino acids, ascorbic acid, total sugars, total soluble solids (TSS), total phenols content (TPC), total flavonoids content (TFC), and nitrate content were enhanced under deficit irrigation conditions. The results showed that under FI or $DI_{40\%}$ with foliar application of SA at 1.0 mM was more effective than 0.5 and 1.5 mM in improving vegetative growth and yield parameters. However, FI produced the highest significant values of plant height, total chlorophyll, fruit yield, carotenoids, and total dry matter. Conversely, the pepper plant's ability to withstand $DI_{40\%}$ was enhanced by the 1.0 mM SA treatment, which resulted in the highest accumulation of ascorbic acid, total sugars, TSS, TPC, and TFC. Moreover, the application of SA significantly increased WUE and decreased fruit nitrate content. It could be concluded that the foliar application of SA can alleviate the detrimental effects of deficit irrigation on pepper plants.

Keywords: drought stress; salicylic acid; sweet pepper; water use efficiency; fruit yield and quality.

1. Introduction

Sweet pepper (*Capsicum annuum* L.) is a popular and widely grown vegetable plant worldwide. Its fruit is a major source of vitamins, minerals, and antioxidants that benefit human health and nutrition (Mateos et al., 2003). Sweet pepper, as a member of the Solanaceae family, is one of the most important vegetable crops and is highly valued in the international marketplace (Salama, 2022; Shedeed et al., 2023).

In arid and semiarid areas of the world, freshwater shortage poses an essential challenge to irrigated crops and food production (Mohammed et al., 2023; Abd El-Aty et al., 2023). With the population growth and increasing effects of climate change, this problem will only worsen in these areas; in particular, the current climate shifts are linked to simultaneous increases in mean and maximum temperatures and low precipitation. Thus, using DI techniques of complete irrigation could be an appropriate strategy to deal with food shortages brought on by the lack of fresh water (El-Hendawy et al., 2017). However, the pepper plants subjected to DI, particularly throughout critical stages of growth, may decrease the dry matter production and fruit yield. Deficit irrigation can have a wide range of detrimental effects on photosynthetic rate, leaf chlorophyll content, and root absorption rate, which can hinder plant growth and development and result in notable reductions in yield (Ghahremani et al., 2021; Namaki et al., 2022; Moustafa et al., 2024). The detrimental effects of DI can be mitigated by applying irrigation water below the total crop water requirements, plus the need for further complementing techniques. Utilizing plant growth regulators at various stages of plant growth may be a valuable strategy to lessen the detrimental effects of abiotic stresses on the yield of horticultural crops (Ennab et al., 2020; Nezamdoost et al., 2023). SA is a plant hormone that belongs to

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phenol compounds that regulate the activation of biotic and abiotic stress defense systems (González-Villagra et al., 2022; El Refaey et al., 2022). In the presence of stress, SA is crucial for controlling the intake of nutrients, cell elongation, levels of photosynthetic pigment, and the activity of photosynthesis, all of which affect the growth and development of plants (Khan et al., 2022; Rashad, 2020). Plants can become more tolerant to abiotic stressors by increasing the efficiency of their metabolic processes with SA (González-Villagra et al., 2022). SA produces these benefits through several processes, including enhanced carbon metabolism, antioxidant system function, cell membrane protection, stress defense, and protein modulation (Sharma et al., 2017). The effects of salicylic acid on plants are contingent upon the method of application, concentration, and stage of plant development (Nóbrega et al., 2020). Numerous studies have examined the functions of SA in enhancing the rate of plant tolerance to abiotic stressors. DI decreased cantaloupe plants' physical characteristics, leaf pigments, components of yield, and overall yield, and they increased even more by adding 0.30 g^{-1} of SA (Nada and Abd El-Hady, 2019). When pepper plants were stressed by drought, SA treatment enhanced the overall yield by controlling stomatal conductance and raising the amount of chlorophyll in their leaves (Ghahremani et al., 2023). Furthermore, using SA externally stimulates plant cells to synthesize and accumulate osmo-protectants, such as proline, which provides defense from abiotic stress (Elhakem, 2020).

Consequently, the main goal of this study was to determine the appropriate foliar application of SA to improve the plant parameters, yield, fruit quality, and water use efficiency of sweet pepper plants under full and deficit irrigation conditions.

2. Materials and Methods

The experiment was conducted for two successive growing seasons, 2021–2022 and 2022–2023, at Al-Sadat farm, Faculty of Agriculture, Al-Azhar University, Menoufia, Egypt (30°23'16.1" N 30°32'06.8" E). The aim is to study the effect of various irrigation rates, foliar application of salicylic acid (SA), and their interaction on the growth and yield of sweet pepper (*Capsicum annuum* L.) under greenhouse conditions. The greenhouse was 40 m in length, 9 m in width, 3.5 m in height, and covered with 200µ thick polyethylene sheets. The experimental soil was categorized as sandy loam. Physical and chemical analysis were conducted of the soil before planting, as reported by Klute (1986) and Sparks et al. (2020), and the results of the analysis are in Table 1. The experiment was carefully prepared by two ploughing, levelling, and separating into experimental plots. Two GR 16 mm diameter drip lines for each row with 0.3 m spacing, 4 L h^{-1} discharge rate, and 1.5 bar operating pressure were employed to apply the irrigation water. Before transplanting, irrigation water was provided to a greenhouse to ensure optimal plant establishment. On September 25th, fifty-day-old sweet pepper seedlings were planted in both seasons. Sweet pepper was transplanted in two lines in each row with a spacing of 25 cm between the two lines in each row and 60 cm between the plants. Throughout the sweet pepper growth stages, the greenhouse's average temperatures and relative air humidity were 31.5 ± 2.1°C and 57 ± 3%, respectively.

2.1 Experimental design and Treatments

The experiments used a split-plot design with three replications. The main plots were given full irrigation (FI= 100% of ETc) and deficit irrigation ($DI_{40\%}$ = 60% of ETc) as irrigation rates. The foliar application with SA at 0.5 (SA1), 1.0 (SA2), and 1.5 mM (SA3), as well as the control (SA0: without foliar application), were given to the sub-plots (Fig. 1). Different concentrations of SA solutions at 0.5, 1.0, and 1.5 mM were prepared by dissolving 69.06, 138.12, and 207.18 mg of SA, respectively, in ethyl alcohol and volume was made up to 1 L with redistilled water. Foliar spraying was done three times; the first was thirty days after transplanting, then repeated every twenty days. Three rows (5.0 m length and 1.2 m width) made up the experimental plot area (6.0 m^2).

2.2 Agricultural practices

All plants received the recommended N, P, and K fertilizers. The fertilizers were directly injected into the irrigation water (fertigation) in two dosages each week, starting from the second week after transplanting until the final harvest, by the recommendations of the Ministry of Agriculture and Land Reclamation. Further agricultural practices were performed when necessary and appropriate for sweet pepper production in the greenhouse.

2.3 Irrigation requirements (IR)

Two rates of irrigation requirements, including FI = 100% and $DI_{40\%}$ = 60% of ETc were used in this experiment. FI = 100% of ETc application is considered full irrigation (no stress), whereas $DI_{40\%}$ = 60% of ETc results in stress treatments. Therefore, the following equation was used to determine the irrigation applied using the drip technique described by Abd El-Mageed et al. (2021):

$$IR = \frac{ETc \times A \times Ii}{Ea \times 1000 \times (1 - LR)}$$

Where:

IR is the irrigation requirements (m^3), ETc is the crop evapotranspiration (mm day^{-1}), A is the area (m^2), Ii is the irrigation interval (day), Ea is the irrigation efficiency (85%), and LR is the leaching requirements (m^3).

Crop evapotranspiration (ETc):

Under plastic greenhouse, the values of ETc were measured according to Doorenbos and Pruitt (1977) by using the equation as follows:

$$ETc\ (mm\ day^{-1}) = ETo \times Kc \times 0.70$$

Where:

ETo is the reference evapotranspiration (mm day^{-1}), whose values are calculated using the Penman-Monteith equation based on daily meteorological data according to Allen et al. (1998). According to Abou Hadid and El-Beltagy (1992), the value of ETo in the plastic greenhouse conditions was almost 70% of the value of ETo in the open field, and Kc is the crop coefficient differing with plant sweet pepper growth stages provided by Allen et al. (1998). Average values of growth stage, reference evapotranspiration (ETo), crop evapotranspiration (ETc), and irrigation requirements (IR) under a greenhouse with both irrigation rates for sweet pepper plants are shown in Table 2.

2.4 Measurements

2.4.1. Plant parameters

Seventy days after transplantation, five plants were selected randomly for each subplot. The plant height was determined by the meter. For chemical analysis, samples were collected from the fourth leaf on the stem. Leaf chlorophyll content was measured using a SPAD chlorophyll meter (Konika Minolta INC, Japan).

Proline content (µg g^{-1} FW) was measured using the colorimetric method at wavelengths of 520 nm by spectrophotometer (6800 UV/Vis Spectrophotometer, Jenway, Bibby Scientific Ltd., Staffordshire, UK), as reported by Bates et al. (1973).

Total free amino acids (mg g^{-1} DW) were determined using the ninhydrin reagent and measured colorimetrically at 570 nm wavelengths by Moore and Stein (1954).

2.4.2. Fruit physical parameters

Ten pickings of the green mature stage were harvested at weekly intervals, and the fruit physical parameters were recorded, including fruit number per plant, fruit length (cm), fruit diameter (cm), and fruit volume (cm^3).

Fruit yield: The yield (ton ha^{-1}) was estimated by computing the data from all pickings during the two seasons.

Water use efficiency (WUE): The WUE (Kg m^{-3}) was estimated by the next formula as mentioned by Zhang (2003):

$$WUE = \frac{\text{Fruit yield (kg ha}^{-1}\text{)}}{\text{Water applied (m}^3\text{ha}^{-1}\text{)}}$$

2.4.3. Fruit chemical parameters

The following parameters were determined by taking random samples from the fourth picking of fruit included:

- Fruit carotenoids (FCar): They were calorimetrically measured (µg g^{-1} FW) using a spectrophotometer according to the protocol described by Lichtenther (1987).
- Total dry matter (TDM): The total dry matter (%) was determined by drying 100 g of fresh fruit in an oven at 70°C until their weight remained constant. Ascorbic acid (ASA): It was determined as mg 100 g^{-1} FW using the methodology of titration with 2, 6-dichlorophenol indophenol (DCPIP), which was outlined by Casanas et al. (2002).
- Total sugars (TS): The ethanolic extract of fresh fruits was used to determine the total sugars using phenol-sulfuric acid as reported by DuBois et al. (1956).
- Total soluble solids (TSS): TSS (%) were measured in the mixed fruit juice using a digital refractometer (38-B1, Bellingham Stanley, UK), which was determined by following the A.O.A.C. (1980).
- Total phenols content (TPC): The TPC was estimated by the Folin-Ciocalteau method as reported in the work of Singleton et al. (1999). In short, 1 ml of Folin reagent (50%) was combined with 1 ml of fruit extract (100 mg with 5 ml methanol 95%). Next, 1 ml of a 10% (w/v) sodium carbonate solution was added. A spectrophotometer was used to measure the absorbance of the mixture at 700 nm after it had been left to stand at room temperature for an hour. The values of TPC were expressed as mg gallic acid equivalent (GAE) g^{-1} DW.
- Total flavonoid content (TFC): The TFC was measured calorimetrically using the aluminium chloride reagent, according to Jagadish et al. (2009). In short, 0.3 ml of 5% (w/v) sodium nitrite solution was combined with 1 ml of fruit extract or various concentrations of quercetin standard solution. The mixture was left to sit at room temperature for six minutes after adding 0.3 ml of 10% (w/v) aluminium chloride.

Table 1. Some physical and chemical properties of experimental soil as an average of two growing seasons 2021–2022 and 2022–2023.

Soil property	Unit	Value	
Particle size distribution:			
Coarse sand	(%)	6.99	
Fine sand		75.45	
Silt		4.86	
Clay		12.70	
Texture class		Sandy loam	
Field capacity	(%)	13.11	
Permanent wilting point		5.77	
Available water		7.34	
Bulk density	(Mg m^{-3})	1.69	
Total porosity	(%)	36.23	
pH (1:2.5 soil water suspension)		8.51	
ECe (soil paste extract, dS m^{-1})	(dS m^{-1})	0.67	
Organic carbon	(g kg^{-1})	2.65	
Organic matter		4.57	
$CaCO_3$		45.15	
Soluble cations (mmolc L^{-1}):		Soluble anions (mmolc L^{-1}):	
Ca^{2+}	1.59	CO_3^{2-}	0.00
Mg^{2+}	1.84	HCO_3^{-}	1.73
Na^{+}	2.86	Cl^{-}	4.34
K^{+}	0.45	SO_4^{2-}	0.67

Table 2. Aggregate monthly amounts of reference evapotranspiration, crop evapotranspiration, and the water requirement applied at full irrigation and deficit irrigation for sweet pepper in both growing seasons 2021–2022 and 2022–2023 under greenhouse conditions.

Months	1st season				2nd season			
	ETo (mm)	ETc (mm)	FI (m^3 ha^{-1})	DI (m^3 ha^{-1})	ETo (mm)	ETc (mm)	FI (m^3 ha^{-1})	DI (m^3 ha^{-1})
Sep. (5 days)	23.23	9.94	146.23	87.74	21.67	9.28	136.45	81.87
Oct. (31 days)	115.78	52.90	777.88	466.73	119.21	54.46	800.93	480.56
Nov. (30 days)	75.45	52.82	776.73	466.04	74.98	52.48	771.81	463.09
Dec. (31 days)	69.97	48.98	720.24	432.14	64.14	44.90	660.27	396.16
Jan. (31 days)	65.71	45.99	676.39	405.83	65.48	45.83	674.01	404.41
Feb. (28 days)	80.34	56.24	827.08	496.25	73.24	51.27	753.92	452.35
Mar. (31 days)	114.67	69.09	1,016.04	609.62	103.28	62.23	915.08	549.05
Total	545.15	335.96	4940.59	2964.35	522.00	320.45	4712.50	2827.50

ETo: reference evapotranspiration; ETc: crop evapotranspiration; FI: full irrigation and DI: deficit irrigation.

After that, add 0.4 ml of 1 M sodium hydroxide (NaOH) came next. After allowing the blend to rest for 12 minutes at ambient temperature, a spectrophotometer was used to measure the absorbance at 510 nm. The TFC was expressed as mg quercetin equivalent (QE) g^{-1} DW.

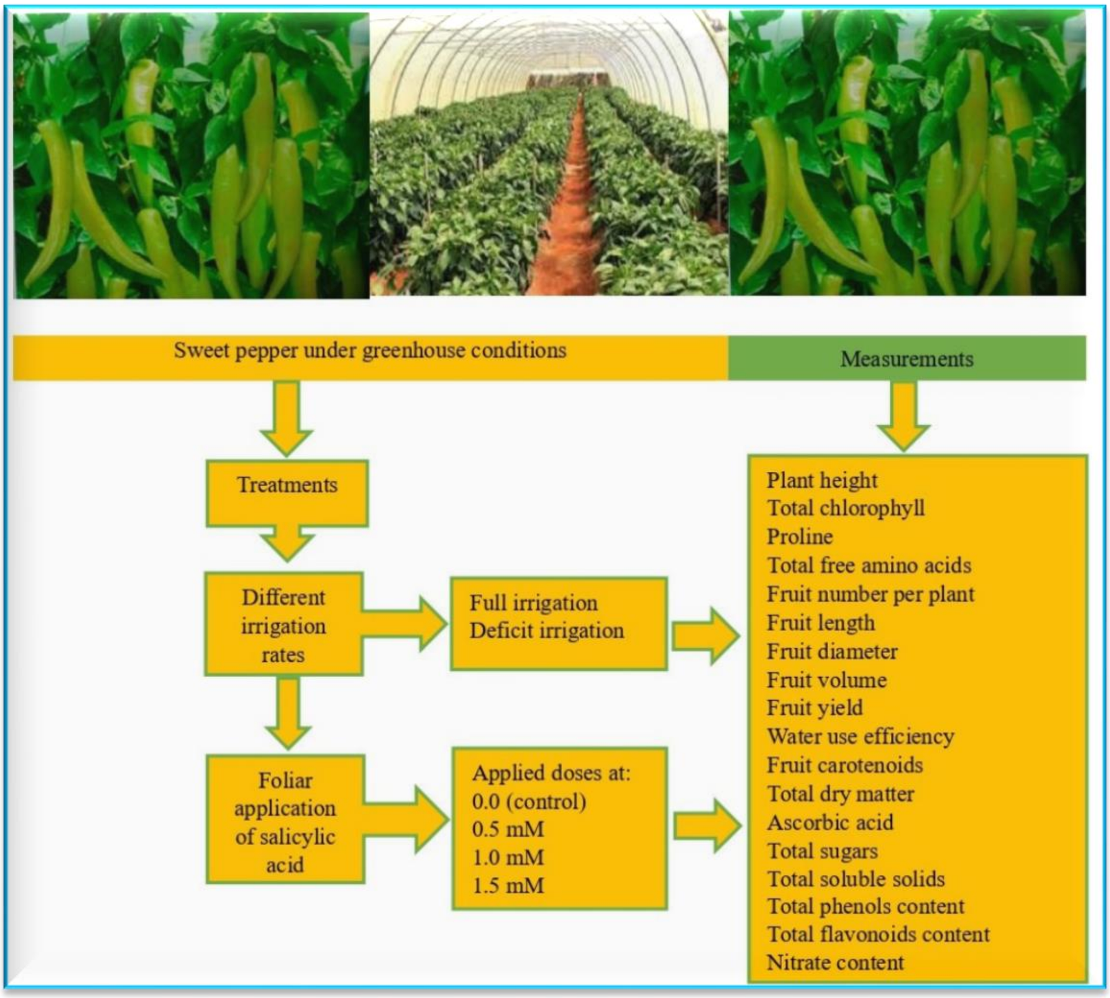

Fig. 1. An overview of all treatments applied in the study, including the different rates applied and the studied variables.

Nitrate content (NC): It was estimated using the method of Cataldo et al. (1975). After homogenizing 100 mg of dried material for an hour in 5 ml of distilled water, the mixture was centrifuged at 4000 rpm for 15 minutes, 0.4 ml of salicylic acid (5% in concentrated sulfuric acid) was added to 0.1 ml of supernatant and thoroughly mixed. 9.5 ml of NaOH 2N were added and combined after 20 minutes. After bringing the mixture to room temperature, the spectrophotometer measured the absorption at 410 nm compared to a blank. The calibration curve was made with KNO_3 dilutions. The nitrate content was stated in mg kg^{-1} DW.

2.5 Statistical analyses

The data were subjected to analysis of variance procedures (ANOVA) using CoStat version 6.4 software (CoHort Software, Monterey, CA, USA). The significant differences between means of treatments were made using Tukey's HSD Multiple Range Test at $P < 0.05$ level (Snedecor and Cochran, 1980). OriginPro 2023 software was utilized to compute correlation using the Pearson test.

3. Results

3.1 Irrigation rates on plant growth and fruits

Based on the data analyzed in this study, the impact of irrigation rates, foliar spraying with salicylic acid, and their interactions on the tested parameters of sweet pepper had significant effects in both seasons with three scenes. The first scene has been cleared in Table 3, which shows trends of decrease and increase by $DI_{40\%}$ in distinctive characteristics. When compared to FI, the $DI_{40\%}$ significantly reduced plant height (22 and 14%), total chlorophyll (10 and 13%), fruit number per plant (17 and 14%), length (9 and 10%), diameter (16 and 16 %), volume (23 and 23%), yield (25 and 15%), carotenoids (12 and 10%) and total dry matter (18 and 13%) in the 1st and 2nd seasons, respectively. On the other side, an increase in proline (49 and 36%), free amino acids (11 and 5%), WUE (25 and 40%), ascorbic acid (18 and 18%), total sugars (38 and 20%), TSS (12 and 20%), total phenols content (18 and 15%), total flavonoids (25 and 14%) and nitrate content (14 and 23%) was noticed in both seasons, respectively, under $DI_{40\%}$ condition.

3.2 Salicylic acid on plant growth and fruits

The second scene was observed with the response of the tested characteristics to different foliar applications of SA alone. General trends in Table 4 show significant increases in the tested characteristics by using 1.0 mM SA, and then a reduction happened by using 1.5 mM of SA, except for the nitrate content parameter. When compared to the control treatment, application of 1.0 mM SA increased the plant height (25.28 and 18.99%), total chlorophyll (14.89 and 20.30%), proline (15.47 and 31.89%), free amino acids (15.40 and 7.70%), fruit number per plant (10.97 and 24.39%), fruit length (17.15 and 20.52%), fruit diameter (19.29 and 30.14%), fruit volume (32.82 and 26.99%), fruit yield (24.23 and 17.66%), WUE (39.81 and 19.40%), carotenoids (5.76 and 7.37%), total dry matter (23.20 and 14.95%), ascorbic acid (27.18 and 18.86%), total sugars (44.98 and 33.25%), TSS (24.68 and 18.67%), TPC (35.35 and 39.71%) and TFC (29.46 and 26.30%), respectively. In comparison, nitrate content (31.60 and 38.37 %) was negatively impacted using SA at 1.0 mM in the 1st and 2nd seasons, respectively.

3.3 Irrigation rates and salicylic acid on plant growth and fruits

The third scene was an interplay between irrigation rates and salicylic acid foliar application, ensuring that the tested parameters significantly responded when the plants were sprayed with varying salicylic acid foliar application under FI and $DI_{40\%}$. However, it was found that 1.0 mM SA was more efficient in all the characteristics under both irrigation conditions. Hence, in Fig. 2, the treatment of FI with 1.0 mM SA foliar application recorded maximum values in plant height (86.97 and 81.42 cm) and total chlorophyll (61.46 and 63.34) in both growing seasons, respectively. On the other hand, when irrigation at $DI_{40\%}$ with 1.0 mM SA were used, the values of proline (50.50 and 55.32 µg g^{-1} FW) and total free amino acids (81.16 and 78.22 mg g^{-1} DW) in the 1st and 2nd seasons, respectively, were comparatively greater.

According to the different interaction effects in Fig. 3, the plants treated with 1.0 mM SA under FI produced the heights significant values of fruit number per plant (70.77 and 80.94 per plant), length (16.87 and 17.25 cm), diameter (3.03 and 2.87 cm) and volume (59.16 and 58.14 cm^3) in the 1st and 2nd seasons, respectively. The lowest significant value was caused by $DI_{40\%}$ without SA.

The notable interaction between salicylic acid and irrigation treatments in Fig. 4 indicates that FI with SA foliar application at 1.0 mM improved fruit yield (61.66 and 59.44 ton ha^{-1}), followed by FI with SA at 1.5 mM (60.14 and 58.04 ton ha^{-1}) in the 1st and 2nd seasons, respectively. At the same time, the reverse tendency was noticed for $DI_{40\%}$ without SA. On the other hand, the WUE values under $DI_{40\%}$ were highest with foliar application of SA at 1.0 mM (1.71 and 1.90 kg m^{-3}) in the 1st and 2nd seasons, respectively.

Data from Fig. 5 show that the amount of fruit carotenoids and dry matter increased when plants were sprayed with 1.0 mM SA, which decreased by 1.5 mM under both irrigation conditions. Thus, the maximum of both contents was found in the treatment of FI plus 1.0 mM SA, i.e., (96.27 and 97.24 µg g^{-1} FW) for fruit carotenoids and (8.61 and 8.08%) for total dry matter, in the 1st and 2nd seasons, respectively. In contrast, the minimum value resulted in $DI_{40\%}$ treatment without SA. Qualitative attributes of pepper fruits, such as ascorbic acid, total sugars, TSS, TPC, and TFC were shown to be significantly impacted by irrigation rates and SA treatments in both seasons (Figures 5 and 6). The interplay between treatments ensured that $DI_{40\%}$ and 1.0 mM SA produced the highest accumulations of this components, with values of 141.83 and 135.67 mg g^{-1} FW for ascorbic acid, 5.99 and 6.05 mg g^{-1} DW for total sugars, 5.36 and 5.52% for TSS, 7.61 and 7.49 mg GAE g^{-1} DW for TPC, and 18.72 and 17.87 mg g^{-1} DW for TFC, in the 1st and 2nd seasons, respectively. Conversely, the least significant effect was caused by FI in the absence of SA. The data presented in Fig. 6 indicate that the interaction effects of irrigation rates and foliar application of salicylic acid on sweet pepper exhibited that the highest significant increases in fruit nitrate content (3.16 and 3.18 mg kg^{-1} DW) resulted from $DI_{40\%}$ without SA in both growing seasons, respectively. At the same time, the lowest values were exerted from FI and 1.0 mM SA.

Table 3. Effect of different irrigation rates on the plant parameters, fruit physical and chemical parameters of sweet pepper in both growing seasons 2021–2022 and 2022–2023.

Traits	1st season		2nd season	
	FI	DI	FI	DI
Plant parameters				
Plant height (cm)	79.17±2.07a	61.85±1.62b	75.62±1.3a	64.76±1.49b
Total Chl. (SPAD)	57.3±0.92a	51.35±1.04b	58.94±1.32a	51.53±0.98b
Proline (μg g^{-1} FW)	31.48±0.51b	46.75±0.99a	35.6±0.97b	48.56±1.51a
TFAA (mg g^{-1} DW)	70.76±1.17b	78.22±0.67a	71.12±0.5b	74.85±0.71a
Fruit physical parameters				
Fruit number per plant	68.14±0.84a	56.87±2.27b	74.86±1.74a	64.51±1.83b
Fruit length (cm)	15.97±0.29a	14.61±0.4b	16.33±0.24a	14.67±0.41b
Fruit diameter (cm)	2.81±0.06a	2.35±0.11b	2.67±0.05a	2.25±0.1b
Fruit volume, (cm^3)	53.72±1.66a	41.38±1.57b	53.13±1.22a	40.8±1.28b
Fruit yield (ton ha^{-1})	56.49±1.47a	42.25±2.02b	57.01±0.72a	48.29±1.35b
WUE (kg m^{-3})	1.19±0.03b	1.49±0.07a	1.25±0.02b	1.76±0.05a
Fruit chemical parameters				
Fruit Car. (μg g^{-1} FW)	93.43±0.6a	82.42±0.8b	94.83±0.59a	85.26±0.81b
Total dry matter (%)	7.97±0.22a	6.75±0.16b	7.68±0.14a	6.8±0.11b
Ascorbic acid (mg g^{-1} FW)	109.8±2.97b	129.4±2.73b	106.9±2.24b	126.0±2.19a
Total sugars (mg g^{-1} DW)	3.95±0.21b	5.45±0.19a	4.45±0.25b	5.34±0.13a
Total soluble solids (%)	4.52±0.11b	5.04±0.09a	4.19±0.08b	5.02±0.1a
TPC (mg GAE g^{-1} DW)	5.69±0.18b	6.72±0.23a	5.82±0.21b	6.72±0.27a
TFC (mg g^{-1} DW)	13.88±0.42b	17.33±0.36a	14.12±0.45b	16.1±0.34a
NC (mg kg^{-1} DW)	2.52±0.11b	2.88±0.06a	2.17±0.18b	2.67±0.09a

Full irrigation (FI), deficit irrigation (DI), total free amino acids (TFAA), water use efficiency (WUE), fruit carotenoids (Fruit Car.), total phenols content (TPC), total flavonoids content (TFC) and nitrate content (NC). Data are means of three replicates ± S.E. Bars with the same letters are not significantly different ($P < 0.05$ level).

Table 4. Effect of foliar application of salicylic acid on the plant parameters, fruit physical and chemical parameters of sweet pepper in both growing seasons 2021–2022 and 2022–2023.

Traits	SA0	SA1	SA2	SA3
1st season				
Plant parameters				
Plant height (cm)	69.42±0.66d	76.39±0.63c	86.97±0.38a	83.89±0.67b
Total Chl. (SPAD)	53.49±0.52d	55.77±0.37c	61.46±0.52a	58.49±0.25b
Proline (µg g^{-1} FW)	29.47±0.22g	31.64±0.24e	34.03±0.13d	30.78±0.06f
TFAA (mg g^{-1} DW)	66.67±0.22g	70.4±0.45e	76.96±0.57c	68.99±0.58f
Fruit physical parameters				
Fruit number per plant	63.77±0.82c	68.61±0.57b	70.77±0.39a	69.41±0.7ab
Fruit length (cm)	14.40±0.17e	16.47±0.27b	16.87±0.12a	16.13±0.03b
Fruit diameter (cm)	2.54±0.02d	2.87±0.07ab	3.03±0.03a	2.80±0.06ab
Fruit volume, (cm^{3})	44.54±0.34e	55.08±0.13c	59.16±0.18a	56.1±0.13b
Fruit yield (ton ha^{-1})	49.63±0.73c	54.53±0.71b	61.66±0.64a	60.14±0.6a
WUE (kg m^{-3})	1.08±0.02d	1.35±0.09c	1.51±0.09a	1.43±0.07b
Fruit chemical parameters				
Fruit Car. (µg g^{-1} FW)	91.02±0.53c	92.68±0.52b	96.27±0.2a	93.76±0.32b
Total dry matter (%)	5.86±0.03e	7.02±0.12cd	7.22±0.08c	6.9±0.09d
Ascorbic acid (mg g^{-1} FW)	93.67±0.88g	110.6±0.69f	119.1±0.44d	115.8±0.73e
Total sugars (mg g^{-1} DW)	3.09±0.09g	4.03±0.09f	4.48±0.04d	4.21±0.08e
Total soluble solids (%)	3.93±0.03f	4.53±0.09e	4.9±0.06cd	4.73±0.07de
TPC (mg GAE g^{-1} DW)	4.78±0.11f	5.81±0.09d	6.47±0.05c	5.68±0.04de
TFC (mg g^{-1} DW)	11.64±0.3g	14.12±0.32f	15.07±0.15e	14.68±0.3ef
NC (mg kg^{-1} DW)	2.88±0.02b	2.59±0.06c	1.97±0.09d	2.65±0.07c
2nd season				
Plant parameters				
Plant height (cm)	63.03±2.95d	70.37±1.84c	75.00±2.88a	72.37±2.11b
Total Chl. (SPAD)	49.11±1.2d	55.77±2.08c	59.08±1.91a	56.98±1.51b
Proline (µg g^{-1} FW)	36.15±2.28c	42.16±2.69b	47.68±3.42a	42.34±3.22b
TFAA (mg g^{-1} DW)	70.21±0.73c	72.69±0.74b	75.66±1.16a	73.4±0.8b
Fruit physical parameters				
Fruit number per plant	61.52±2.55d	67.04±2.07c	76.53±2.01a	73.65±2.74b
Fruit length (cm)	13.79±0.61c	15.68±0.33b	16.62±0.3a	15.93±0.29b
Fruit diameter (cm)	2.09±0.16c	2.49±0.09b	2.72±0.08a	2.55±0.08ab
Fruit volume (cm^{3})	40.45±3.05d	48.65±2.88b	51.37±3.04a	47.39±2.09c
Fruit yield (ton ha^{-1})	47.5±2.65c	52.08±2.3b	55.89±1.62a	55.14±1.35a
WUE (kg m^{-3})	1.34±0.08c	1.48±0.10b	1.60±0.14a	1.59±0.14a
Fruit chemical parameters				
Fruit Car. (µg g^{-1} FW)	86.53±2.35d	89.99±2.3c	92.91±1.94a	90.75±1.99b
Total dry matter (%)	6.62±0.16c	7.22±0.23b	7.61±0.22a	7.52±0.20a
Ascorbic acid (mg g^{-1} FW)	105.2±4.74d	116.6±3.29c	125.1±4.74a	118.9±4.35b
Total sugars (mg g^{-1} DW)	4.3±0.27d	4.68±0.41c	5.73±0.15a	4.88±0.05b
Total soluble solids (%)	4.23±0.18c	4.56±0.15b	5.02±0.23a	4.6±0.19b
TPC (mg GAE g^{-1} DW)	4.96±0.16c	6.36±0.17b	6.93±0.26a	6.83±0.27a
TFC (mg g^{-1} DW)	13.57±0.58c	14.77±0.43b	17.14±0.34a	14.95±0.45b
NC (mg kg^{-1} DW)	3.05±0.05a	2.53±0.06b	1.88±0.11d	2.23±0.25c

SA0, SA1, SA2, and SA3: foliar application of salicylic acid at 0.0 (control), 0.5, 1.0, and 1.5 mM, respectively. Data are means of three replicates ± S.E. Bars with the same letters are not significantly different (*P* < 0.05 level).

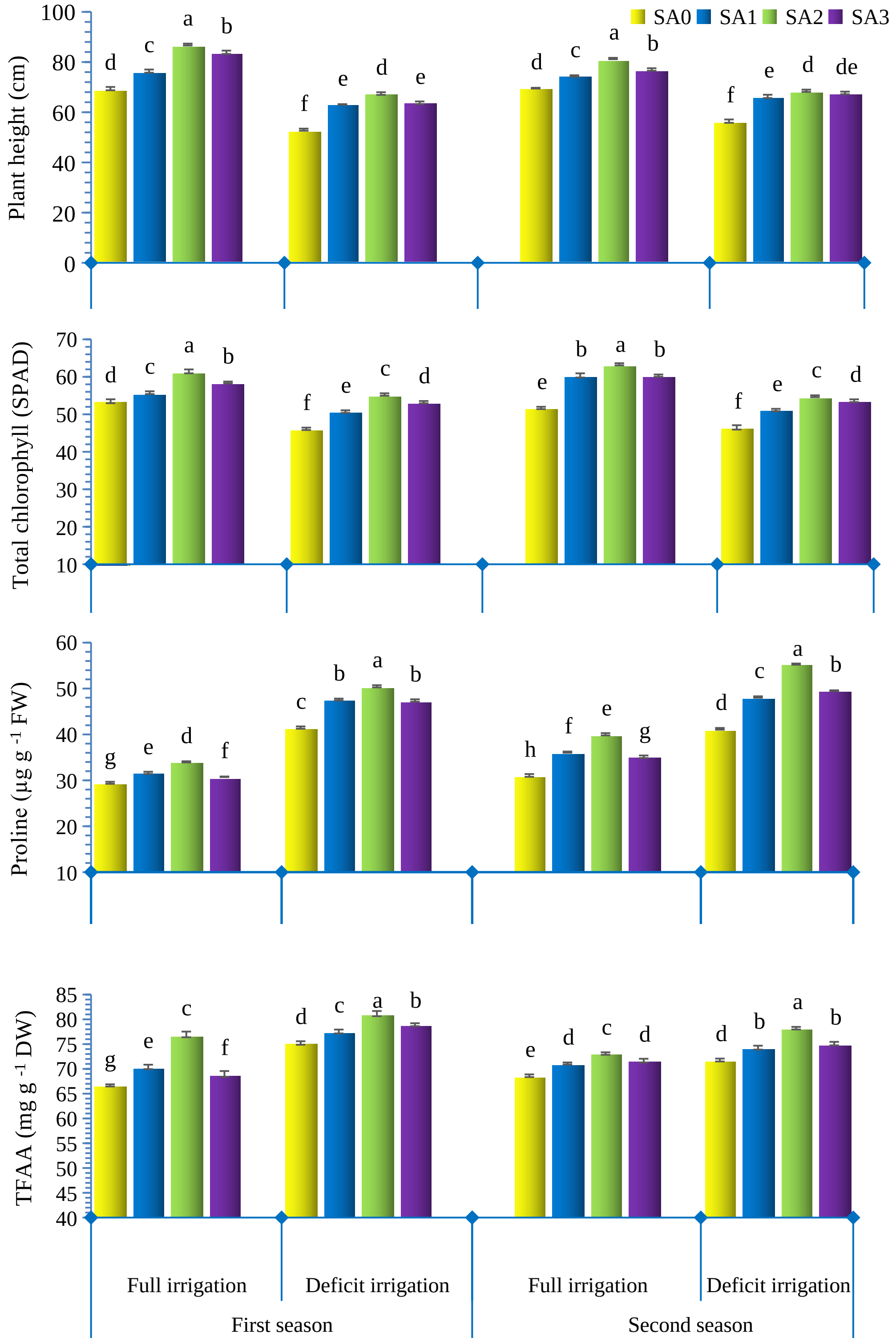

Fig. 2. Effect of the interaction between different irrigation rates and foliar application of salicylic acid on plant height, total chlorophyll, proline, and total free amino acids (TFAA) in sweet pepper in both growing seasons 2021–2022 and 2022–2023. SA0, SA1, SA2, and SA3: foliar application of salicylic acid at 0.0 (control), 0.5, 1.0, and 1.5 mM, respectively. Bars represent ± S.E. Bars with the same letters are not significantly different (*P* < 0.05 level).

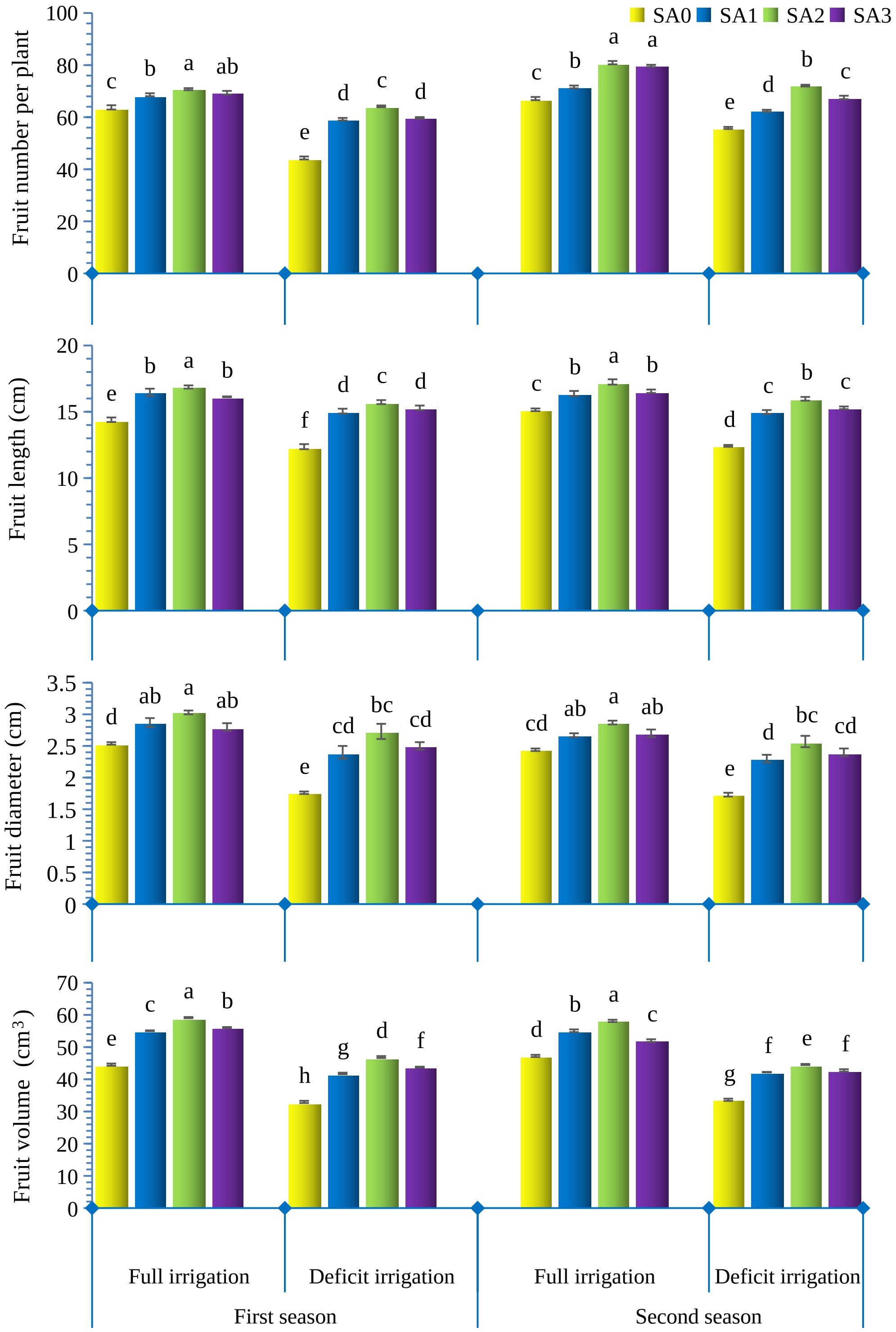

Fig. 3. Effect of the interaction between different irrigation rates and foliar application of salicylic acid on fruit number per plant, fruit length, fruit diameter, and fruit volume of sweet pepper in both growing seasons 2021–2022 and 2022–2023. SA0, SA1, SA2, and SA3: foliar application of salicylic acid at 0.0 (control), 0.5, 1.0, and 1.5 mM, respectively. Bars represent ± S.E. Bars with the same letters are not significantly different (P < 0.05 level).

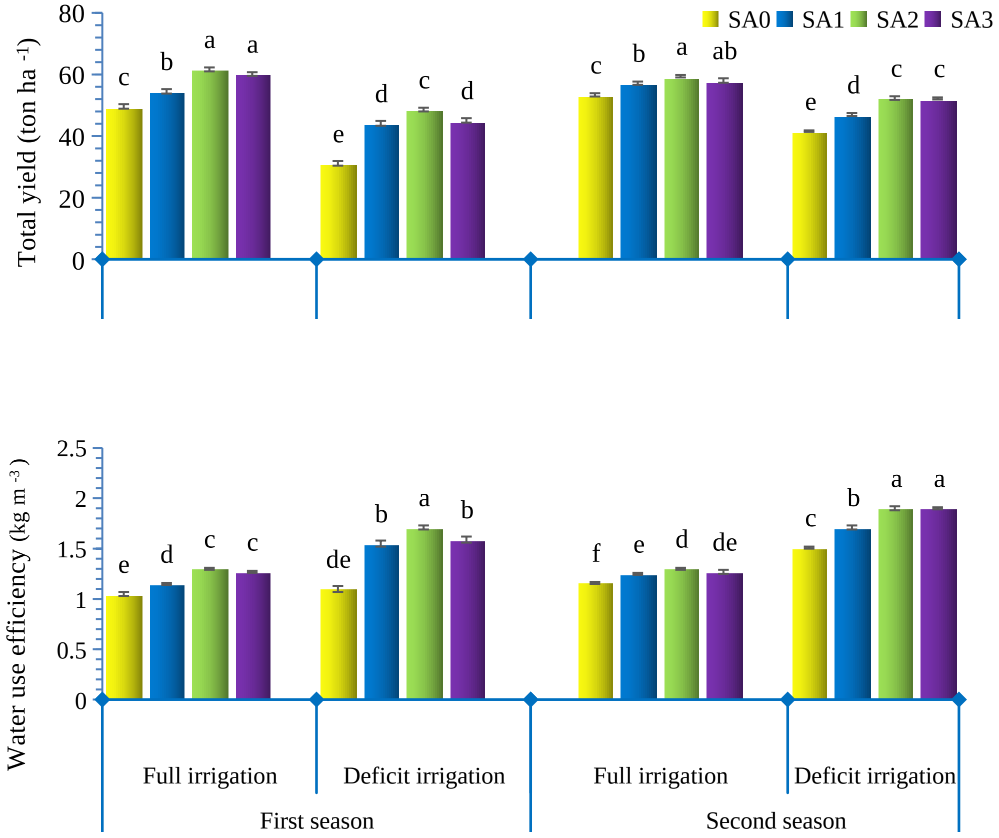

Fig. 4. Effect of the interaction between different irrigation rates and foliar application of salicylic acid on fruit yield and water efficiency of sweet pepper in both growing seasons 2021–2022 and 2022–2023. SA0, SA1, SA2, and SA3: foliar application of salicylic acid at 0.0 (control), 0.5, 1.0, and 1.5 mM, respectively. Bars represent ± S.E. Bars with the same letters are not significantly different ($P < 0.05$ level).

3.4. Pearson correlation analysis

The average various sweet pepper attributes during the two seasons under FI and $DI_{40\%}$ conditions showed significant relationships (positive or negative) according to the Pearson correlation analysis, as shown in Fig. 7. There is a relationship between the observed attributes, with blue and red colors denoting negative and positive correlations, respectively. It was found that fruit yield positively correlated ($P \leq 0.01$) with plant height, total chlorophyll, fruit number per plant, length, diameter, volume, carotenoids, and total dry matter. The positive correlation ($P \leq 0.01$) for TPC and TFC were observed with proline, total free amino acids, WUE, ASA, TS, and TSS. Conversely, a negative correlation ($P \leq 0.01$) was noted between fruit nitrate content and plant height, total chlorophyll, fruit number per plant, length, diameter, volume, and total dry matter. Moreover, a negative correlation at $P \leq 0.05$ was produced between nitrate content and fruit carotenoids.

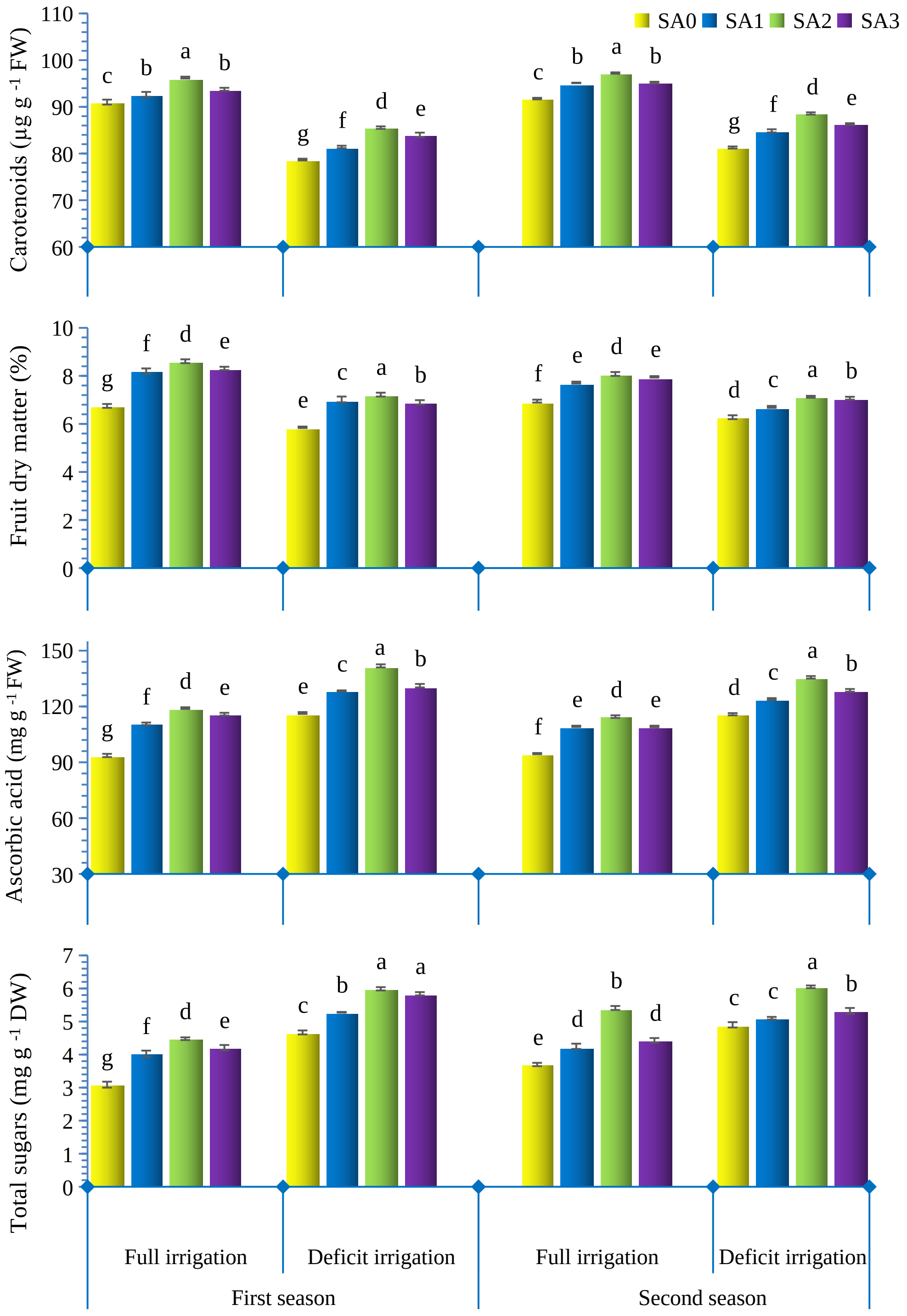

Fig. 5. Effect of the interaction between different irrigation rates and foliar application of salicylic acid on fruit carotenoids, fruit dry matter, ascorbic acid, and total sugars in sweet pepper in both growing seasons 2021–2022 and 2022–2023. SA0, SA1, SA2, and SA3: foliar application of salicylic acid at 0.0 (control), 0.5, 1.0, and 1.5 mM, respectively. Bars represent ± S.E. Bars with the same letters are not significantly different ($P < 0.05$ level).

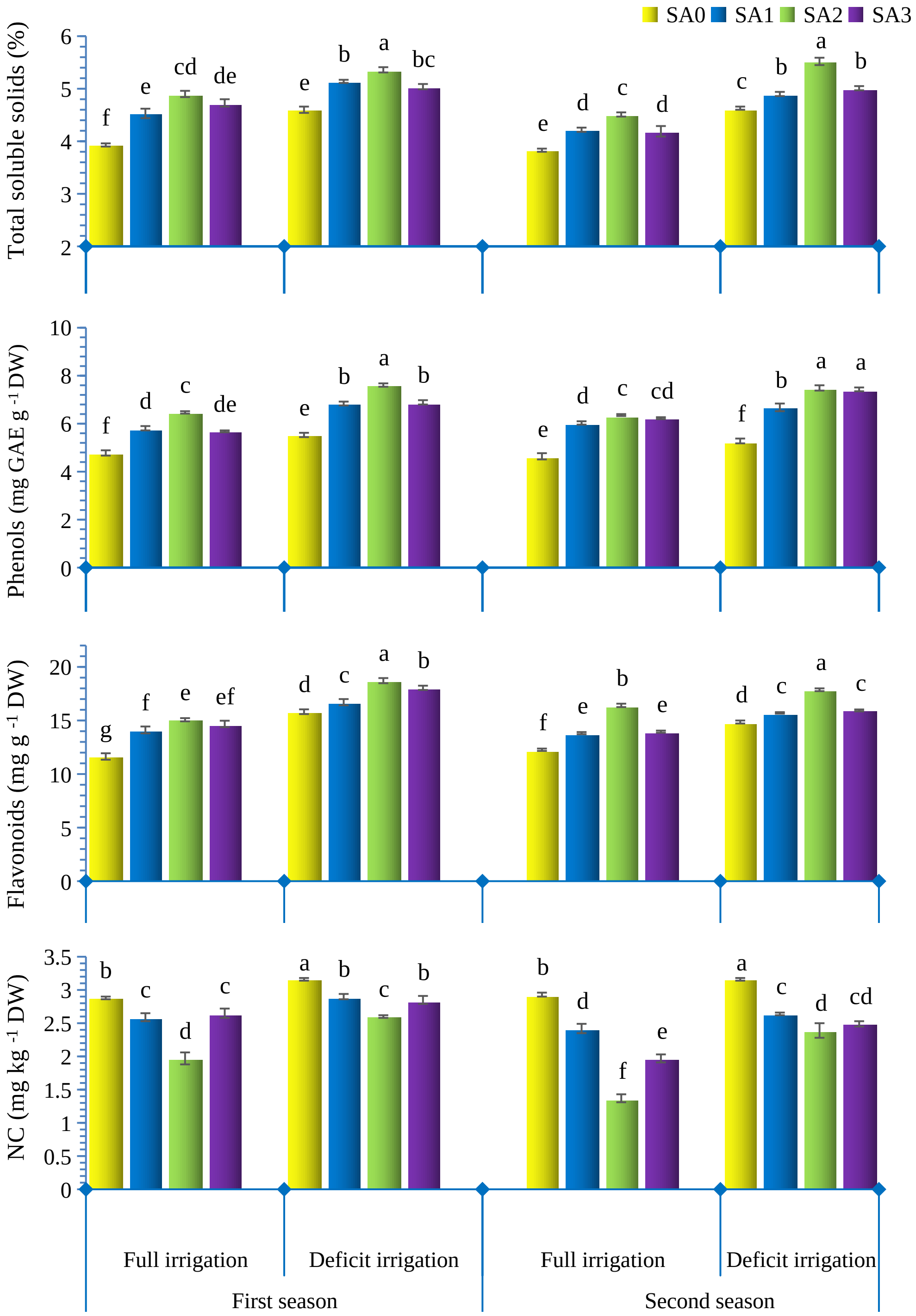

Fig. 6. Effect of the interaction between different irrigation rates and foliar application of salicylic acid on fruit total soluble solids, total phenols content, total flavonoids content, and nitrate content (NC) of sweet pepper in both growing seasons 2021–2022 and 2022–2023. SA0, SA1, SA2, and SA3: foliar application of salicylic acid at 0.0 (control), 0.5, 1.0, and 1.5 mM, respectively. Bars represent ± S.E. Bars with the same letters are not significantly different (*P* < 0.05 level).

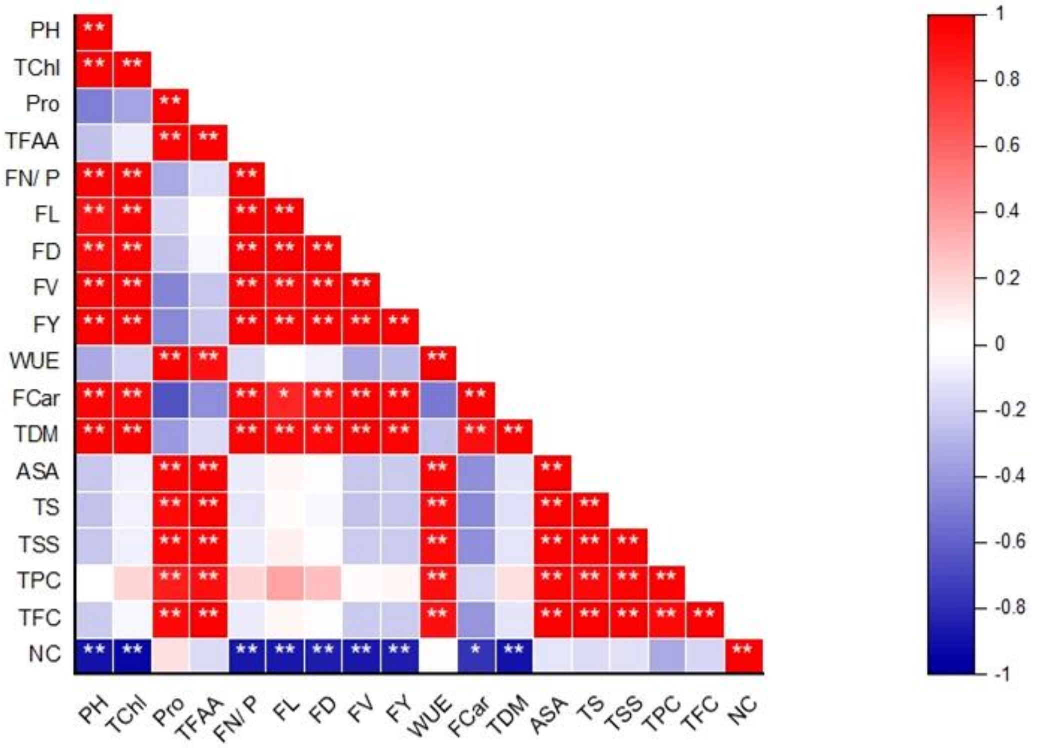

Fig. 7. Pearson correlation analysis between different variables of sweet pepper plants. Plant height (PH), total chlorophyll (TChl), proline (Pro), total free amino acids (TFAA), fruit number per plant (FN/ P), fruit length (FL), fruit diameter (FD), fruit volume (FV), fruit yield (FY), water use efficiency (WUE), fruit carotenoids (FCar), total dry matter (TDM), ascorbic acid (ASA), total sugars (TS), total soluble solids (TSS), total phenols content (TPC), total flavonoids content (TFC) and nitrate content (NC).

4. Discussion

Sweet pepper subjected to $DI_{40\%}$ regularly during growth stages produced decreases in plant height, total chlorophyll, fruit number per plant, length, diameter, volume, yield, carotenoids, and total dry matter compared to FI. However, this was not true for WUE, proline, free amino acids, ascorbic acid, total sugars, TSS, TPC, TFC, and nitrate content. The significant reductions in plant height, total chlorophyll, and fruit physical traits may be due to the sweet pepper under DI, with a 40% reduction at the active root zone. DI conditions are recognized to induce a wide range of molecular, biochemical, and physiological changes that impact the growth and development of plants (Khapte et al., 2019). When plants suffer from DI, their growth may decline for two reasons: either their cells elongate less because of the inhibiting effect of water shortage on growth-promoting hormones, or their xylem and phloem vessels become blocked, which prevents water flow and translocation. These factors reduce cell turgor, volume, and growth (Abdelaal, 2015). Furthermore, DI suppresses growth parameters by lowering the relative water content of leaves, which reduces their turgor and interferes with their ability to assimilate water and nitrogen compounds, affecting cell division and enlargement (Eltarabily et al., 2019). A reduction follows this in root formation and nutrient uptake, reducing the Fe and Mg nutrients supplied to leaves. Both are necessary for synthesizing chlorophyll pigments (Farouk and Ramadan, 2012).

Reducing chlorophyll content may result from increased pigment degradation or impair the pigment biosynthesis process (Nematpour et al., 2020). Thereby, conditions characterized by water stress at $DI_{40\%}$ result in a significant reduction of plant photosynthetic efficiency (Akács et al., 2020), and this is primarily because of stomata closing (Putti et al., 2023), which restricts CO_2 diffusion into the leaf, Rubisco suppression and ATP synthesis reduction (Murtaza et al., 2016). So, probable causes for our findings include a decline in nutrient uptake and inadequate photosynthate accumulation, allowing for a decrease in fruit number per plant, length, diameter, and volume.

A comparable pattern was noted for fruit yield (tonnes per hectare) since fruit weight is a significant factor in the overall pepper yield. (Chen et al., 2016; Javadi et al., 2017; Aghaie et al., 2018). Also, DI contributes to the accumulation of reactive oxygen species (ROS) that cause oxidative damage to cell components like protein, phospholipids, DNA, and RNA, negatively affecting plant growth and yield (ALKahtani et al., 2020). Similarly, there is a decline in fruit carotenoid content response to $DI_{40\%}$. Other studies suggested that the reduction of fruit carotenoids is correlated strongly with chlorophyll degradation (Khazaei et al., 2020). In addition, $DI_{40\%}$ treatment caused a decrease in total dry matter. This could have resulted from a drop in chlorophyll content and, in turn, a reduction of photosynthetic efficiency (Bakry et al., 2012). Thus, the results of our experiment showed a significant correlation between vegetative growth and fruit yield under FI and $DI_{40\%}$ conditions.

Our findings revealed that stressed pepper plants at $DI_{40\%}$ conditions had significantly higher proline and free amino acids than FI. Many reports proved that proline and other amino acids function as osmoregulators in drought-tolerant plant species under stress conditions (Gupta et al., 2020; Ghaffari et al., 2021). Proline and free amino acids were found to regulate cell osmotic potential to enhance water flow under $DI_{40\%}$ and guard the apparatus of photosynthetic membrane enzymes against oxidative harm (Esmaeilpour et al., 2016). Proline is essential for controlling mitochondrial activity, saving chloroplasts from damage caused by oxidation, and causing the expression of genes that aid in plant stress tolerance (Abdelaal et al., 2021). Hence, our results are consistent with earlier findings on pepper, where plants responded to DI by accumulating proline (Khazaei et al., 2020; Mahmood et al., 2021).

Fruit ascorbic acid, total sugars, TSS, TPC, and TFC can be used to evaluate the effects of DI. According to this study, $DI_{40\%}$ treatment improves the previous parameters. Ascorbic acid is a major antioxidant found in plants essential for defending against various environmental abiotic stressors (Arab et al., 2022). During the metabolic process, ascorbic acid in pepper plants can regulate the production of free radicals, enhancing the plant's tolerance against stress and mitigating the adverse impacts of ROS (Khazaei et al., 2020). Our findings indicated that $DI_{40\%}$ caused an increase in the total sugar content and TSS of pepper fruit. TSS is a crucial component of fruit quality, and it measures the mass ratio of the fruit-dissolved sucrose to water. The study's findings suggested that lowering the water used to irrigate the pepper plant decreased the fruit moisture content, raising the TSS. The explanation of how DI can enhance fruit quality says that when pepper plants receive less water during irrigation, they regulate specific metabolic processes, such as osmotic adjustment in sink organs, to increase the rate and amount of sucrose and organic acid transformation. As a result, more assimilates shift to the fruits, improving their soluble sugar and TSS (Agbemafle et al., 2014).

Moreover, a correlation between starch degradation into glucose and increased soluble sugar concentration under water stress was previously reported by Thalmann and Santelia (2017). The rise in soluble sugars is crucial for preserving cellular turgor pressure and the osmotic potential (Arbona et al., 2013).

Total phenols and flavonoids are crucial secondary metabolites that enhance plants' ability to withstand stressful conditions (Ghorbani et al., 2018). In this investigation, $DI_{40\%}$ led to a significant increase in total phenols and total flavonoids. The findings align with prior research indicating that water deficits increased TPC and TFC (Saheri et al., 2020). Also, the results reported that the $DI_{40\%}$ significantly increased nitrate content, which may be linked with nitrate reductase activity (Elwan and Elhamahmy, 2015).

Thus, the projected result under $DI_{40\%}$ conditions is a decline in sweet pepper growth, fruit yield, and physical and chemical parameters. Implementing $DI_{40\%}$ on sweet pepper was challenging without causing a corresponding decrease in growth and yield. Additional treatments are needed for sweet pepper production under water shortage to lessen the adverse effects of water shortage on sweet pepper growth and production. Foliar application with SA is an effective and straightforward method of reducing the negative impacts of water-deficit stress on vegetable growth and productivity. This could be because the exogenous administration of SA is crucial in regulating and activating various biochemical and physiological processes that manage sweet pepper responses to both DI and FI conditions (Khan et al., 2022).

In this investigation, treatments via SA by foliar application up to 1.0 mM demonstrated statistically significant increases in all parameters compared to the untreated plants without SA. Also, this study showed that, under FI or DI conditions, foliar application of SA up to 1.0 mM was generally more effective than 0.5 and 1.5 mM SA in improving growth, physiological, and yield-related parameters. However, FI produced the highest significant values of plant height, total chlorophyll, fruit number per plant, length, diameter, volume, yield, carotenoids, and total dry matter. Generally, these results may be due to the origin of SA as a phenol-based phytohormone that is essential for improving relative water content, nutrient absorption, stomatal opening, photosynthetic activity, cell division, cell elongation, and chlorophyll pigment content (Khan et al., 2022). It integrates into processes for the growth and development of a plant, delays organ ageing, regulates the source-to-sink connection, and participates in specific physiological reactions about absorption of carbon or fixes in the chloroplasts, as well as rubisco activity and concentration (Khalvandi et al., 2021). According to Osama et al. (2019), SA regulatory function is linked to preventing auxin and cytokinin levels from falling, which promotes better cell division in the root apical meristem and enhances plant growth, thus increasing yield.

Furthermore, considering that SA contributes to the production of antioxidants, promotes osmotic adjustment, and scavenges ROS. These processes safeguard the stability of membranes and photosynthesis pigments, improving plant growth characteristics, especially under DI (Pal et al., 2016). Using SA increased the amount of photo-assimilates in the fruits, enhancing their yield marketability and lessening the stress of the water deficit and flower fall (Aires et al., 2022). Further investigations found that SA foliar spray at 0.05 mM increased tomato fruit yield under normal conditions (Sariñana-Aldaco et al., 2020) and that 0.1 mM SA was a practical level to increase the yield of cucumbers (Preciado-Rangel et al., 2019). Exogenous SA application efficacy depends on several factors, including plant species, concentration, and environmental conditions (Poór et al., 2019).

In our study, when $DI_{40\%}$ and SA at 1.0 mM were used, the proline and total free amino acids values were comparatively greater. Proline levels increased following the foliar application of SA because the proline-degrading enzyme proline oxidase's activity decreased, and the proline-synthesizing enzyme Gamma glutamyl kinase's activity increased (Idrees et al., 2010). Our results showed that WUE values under $DI_{40\%}$ were highest, with the SA presence at 1.0 mM or 1.5 mM SA. Improved WUE results from SA ability to increase CO_2 availability during photosynthesis, improve leaf diffusive resistance, and reduce transpiration rate via controlling stomatal behaviour (Munsif et al., 2022).

The interplay between treatments ensured that $DI_{40\%}$ and 1.0 mM SA produced the highest accumulations of ascorbic acid, total sugars, and TSS. The increased ascorbic acid content in pepper fruits treated with foliar application of SA may be because SA effectively promotes the synthesis of carbohydrates, which are necessary precursors for the synthesis of ascorbic acid (Sariñana-Aldaco et al., 2020). At the same time, SA increases TSS in fruits by accumulating carbohydrates and pigments (Youssef et al., 2017).

An analogous pattern was observed for the phenol compounds, with $DI_{40\%}$ and 1.0 mM SA encouraging the highest values. SA promotes the synthesis of antioxidant compounds like phenols by increasing the activity of the phenylalanine ammonium lyase, which oversees the production of these compounds (Aghdam et al., 2012). On the other hand, SA application at 1.0 mM decreased fruit nitrate content under irrigation conditions; however, the lowest values were exerted from FI with 1.0 mM SA. This decrease was linked to nitrate reductase activity (Elwan and Elhamahmy, 2015). The Pearson correlation analysis demonstrated a positive correlation between fruit yield, plant height, total chlorophyll, fruit number per plant, length, diameter, volume, carotenoids, and total dry matter.

Conclusions

The study's findings demonstrated that the most efficient foliar application of SA concentration was 1.0 mM, which may be utilized as a strategy to mitigate the adverse effects of deficit irrigation on the morpho-physiological traits and yield of sweet pepper plants grown under the experiment conditions, where water shortage is one of the main factors limiting the production of the sweet pepper plants.

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