The Role of Sorbitol in Reducing Water Stress on Strawberry Plants

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Abstract
The present investigation was undertaken within unheated, protected greenhouse facilities at the Department of Horticulture and Landscape, College of Agriculture, Tikrit University, Iraq, spanning the 2023–2024 growing seasons. The research objective centered on elucidating the protective capacity of sorbitol in mitigating water.deficit.induced impairment of vegetative growth and productivity in the Ruby Gem cultivar (Fragaria x ananassa). A factorial arrangement within a randomized complete block design, replicated three times, was employed, incorporating two experimental factors: sorbitol application at concentrations of 0, 20, and 40 g L^{-1}, alongside water.stress intervals of 3, 5, and 7 days. Parameters evaluated encompassed fruit number, individual fruit mass, and foliar chlorophyll concentration, in addition to fruit anthocyanin, phenolic, and flavonoid contents.
The results showed significant effects of both sorbitol and water stress, and their interaction on most of the studied traits. The treatment with Sorbitol at 40 g/L^{-1} (S_2) significantly increased the number of fruits, fruit weight and chlorophyll content. In addition, accumulation of anthocyanins, phenols, and flavonoids were increased compared to the control treatment. The results also showed that increased water stress intensity led to decrease in the number and weight of fruits and chlorophyll content. However, it contributed to increases of anthocyanins, phenols and flavonoids as a defensive response. The interaction between the two factors resulted in increases in most of the studied chemical properties; this indicated the positive role of sorbitol in enhancing the ability of strawberry to withstand water stress.

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1. Introduction

The strawberry (Fragaria x ananassa) is a herbaceous perennial, one of the genus Fragaria, in the family Rosaceae and order Rosales, and remarkably ecologically plastic with a wide range of climatic adaptations, having been extensively cultivated and is widely naturalised in North America (Al.Saidi, 2000; Owain *et al*., 2023) [2, 9]. Worldwide production of this crop increased from 4,178,125 million tons in 2009 to 4516,810 million tons in 2012 which was cultivated in an area of 241,109 hectares (Suvalaxmi Palei *et al*., 2015) [15]. This upward trend in the global consumption is mainly due to the antimicrobial and antifungal properties of the fruit, attributed to its phenolic composition, but also to its nutritional profile and palatability, which is rich in lipids, carbohydrates, proteins and minerals such as magnesium, calcium, phosphorus, potassium, copper, zinc and vitamin C (Fierascu *et al*., 2020) [4].

Sorbitol is one of seventeen types of sugar alcohols found in higher plants, It is produced industrially from glucose sugar, and is also found naturally in apricots, pears, cherries, and others. It is one of the types of sugar alcohols, of which there are more than seventeen types found in higher plants. Sorbitol is of great importance in the plant's response to abnormal conditions and stresses such as drought, low temperature, and salinity (Yassin Hassan *et al*., 2023) [16]. Sorbitol plays an osmotic role in maintaining water balance within cells and is used as an energy source and for transporting carbon to vegetative growing parts.

Research has indicated that its addition in tissue culture plays a role in improving vegetative and root growth. It also promotes the accumulation of secondary compounds such as phenols and flavonoids and acts as a regulator of growth signals by stimulating gene expression for adventitious root formation and increasing the activity of growing tips, this makes it effective in micro propagation techniques (Stasolla *et al*., 2008) [14].Water stress is one of the abiotic stress.causing plants that are affected by their natural appearance due to the influence of climate, but with a decrease. increase in irrigation water or a rise. decrease in temperature (Khadir *et al*., 2021) [5]. One of the reasons for the plant being affected by water stress is a decrease in the plant's fresh weight, which leads to a decrease in the dry weight of the shoot and root system compared to a plant that has not been exposed (Parviz *et al*., 2014) [10]. Water stress also causes a decrease in plant height and the formation of undesirable and deformed fruit (Shima *et al*., 2014) [13].

2. Material and Method

The experiment was carried out in one of the unheated protected plastic greenhouses belonging to the Horticultural Facility Unit at the College of Agriculture, Tikrit University, for the 2023.2024 agricultural season, to study the role of sorbitol as a protective substance in reducing water stress on strawberry plants.

2.1. Soil Analyses

Four samples were taken from different locations of the greenhouse soil after the plowing process to conduct chemical and physical analyses. They were analyzed in the soil and water laboratories at the College of Agriculture, Tikrit University. Table No. (1) shows the results of the analyses.

Table 1: shows the physical and chemical properties of the greenhouse soil.

Soil physical and chemical Values properties	Values
Sand %	32
Silt %	25
Mud %	43
Soil pH	7.1
Electrical Conductivity (EC) (dm.1.25°C)	3.45
Organic matter (g.kg)	1.88
Nitrate (NO3) (mg.kg.1)	12.1
Ammonium (NH4) (mg.kg.1)	12.9
Potassium (mg.kg.1)	4.2
Phosphorus (mg.kg.1)	10.3

2.2. Preparing and Planting Seedlings

Strawberry seedlings of the Ruby Gem variety, a short.day variety, were obtained from a specialized strawberry propagation nursery in Sulaymaniyah Governorate. Pruning and removal of foliage were carried out before planting, leaving 1.2 leaves per plant. All seedlings were then planted in an unheated greenhouse in the early morning of November 1, 2024, at a spacing of 30 cm between plants, and watered. All flowers were removed to encourage root and vegetative growth and to prepare the plants for spring planting. Measurements were taken and samples were analyzed.

2.3. Experimental Design and Treatments

The experiment was conducted as a factorial experiment using a completely randomized block design, with three blocks and two factors:

1. The first factor is the addition of a sugar alcohol (sorbitol) as follows:

- Without addition, denoted as S_0.
- 20 g/L^{-1} S_1.
- 40 g/L^{-1} S_2.

2. The second factor is the application of water stresses through irrigation periods, as follows:

- Water every 3 days W_1.
- Water every 5 days W_2.
- Water every 7 days W_3.

2.4. The Characteristics Studied

2.4.1. Average Number of Fruits (fruit. plant.^{1})

Fruit counts were recorded continuously from the initial to the final harvest across five designated plants per experimental unit, with the per.plant average subsequently derived through the following relation:

Average number of fruits per plant.^{1} = Total number of fruits for the five plants / 5.

2.4.2. Average Fruit Weight (g)

The aggregate fruit mass from the five tagged plants was determined and subsequently divided by the corresponding total fruit count to yield the mean fruit weight, expressed as:

Average weight of fruit= Total weight of the fruit of the five plants / Total number of fruits for the five plants.

2.4.3. Leaf Chlorophyll Content (mg/100g soft weight.[1])

Chlorophyll pigments were isolated from fresh leaf tissue following the protocol of Arnon (1949), employing an 80% acetone solvent. Absorbance readings of the resulting extract were subsequently obtained at 645 nm and 663 nm to quantify chlorophyll concentration (mg L^{-1}), applying the equation:

Total Chlorophyll(μgml−1)=$20.2_{A645}+8.02_{A663}$.

2.4.4. Anthocyanin Pigment (mg/100g.[1] soft weight)

The anthocyanin pigment content of the fruits was measured as reported in Ranganna (1977).

2.4.5. Phenol Content Is Estimated in mg/g $^{-1}$

The total phenol content of the methanolic extract was determined using the method described by Makkar *et al.* (1997) with Follin's reagent. Absorbance was recorded at 725 nm using optical absorption spectroscopy, compared to a Planck sample containing only the reagent. The total phenol content was calculated based on the calibration curve.

2.4.6. Estimation of Flavonoids in mg/g [1]

The total flavonoid content was estimated using the Ordonez (2006) method with aluminum chloride, and the absorbance was measured at a wavelength of 420 nm using optical absorption spectroscopy. The total flavonoid content was calculated using a calibration curve.

3. Results and Discussion

3.1. Results

3.1.1. Effect of Sorbitol, Water Stress and The Interaction Between Them on The Average Number of Fruits (Fruit/Plant.1)

The data in Table (1) indicate that there are significant differences between the sorbitol treatments, as treatment S_2 was significantly superior, giving the highest rate of 10.050 fruits.plant^{-1}, followed by treatment S1, which recorded a rate of 9.734 fruits.plant^{-1}, while treatment S_0 gave the lowest rate of 9.096 fruits.plant^{-1}.

As for the effect of water stress, the results showed significant differences between the levels, with level W_0 significantly outperforming, recording the highest rate of 10.435 fruits.plant^{-1}, followed by level W1 with a rate of 9.682 fruits.plant^{-1}, while level W_2 recorded the lowest rate of 8.763 fruits.plant^{-1}.

Regarding the interaction between sorbitol and water stress, the data in the table indicate that the interaction between S2 and W0 gave the highest significant value of 10,500 fruits/plant^{-1}, and did not differ significantly from some other treatments that shared the same letter, while the interaction between S0 and W2 recorded the lowest significant value of 7,440 fruits/plant^{-1}, which indicates the negative effect of increasing water stress in reducing the number of fruits, especially when sorbitol was not added.

Table 2: Influence of sorbitol application, water.stress duration, and their interaction on fruit number (fruit·plant^{-1}).

Sorbitol Rate	W0	W1	W2	Mean (Sorbitol)
S0	10.433a	9.416b	7.440c	9.096b
S1	10.373a	9.446b	9.383b	9.734a
S2	10.500a	10.183a	9.466b	10.050a
Mean (Water Stress)	10.435a	9.682b	8.763c	

Means sharing identical letters within the table are not significantly different, as determined by Duncan's multiple range test at the 0.05 probability level.

3.1.2. Effect of Sorbitol and Water Stress on Average Fruit Weight (G)

The data in Table (2) indicate that there are significant differences between the sorbitol acid treatments, as treatments S_2 and S1 were significantly superior, giving the highest rates of 18.273 and 18.133 g respectively, with no significant differences between them, while treatment S_0 recorded the lowest rate of 17.661g.

As for the effect of water stress, the results showed significant differences between the levels, with level W_0 significantly outperforming, recording the highest rate of 18.574g, followed by level W_1 with a rate of 18.195g, while level W_2 gave the lowest rate of 17.297g.

Regarding the interaction between sorbitol acid and water stress, the table data indicates that the interaction between S_2 and W_0 yielded the highest significant value of 18.856g/fruit^{-1}, and did not differ significantly from some treatments sharing the same letter, such as S1 with W_0 and W_1. Meanwhile, the interaction between S0 and W2 recorded the lowest significant value of 16.416g/fruit^{-1}, indicating the negative impact of high-water stress on reducing fruit weight, especially when sorbitol acid was not added.

Table 3: Effect of sorbitol and water stress on average fruit weight (g):

Sorbitol Rate	W0	W1	W2	Mean (Sorbitol)
S0	18.376ab	18.190b	16.416d	17.661b
S1	18.490ab	18.450ab	17.460c	18.133a
S2	18.856a	17.946bc	18.016b	18.273a
Mean (Water Stress)	18.574a	18.195b	17.297c	

Mean values bearing the same letter designation within the table do not differ significantly from one another, based on Duncan's multiple range test at a 0.05 probability threshold.

3.1.3. Effect Of Sorbitol And Water Stress On Leaf Chlorophyll Content (Mg.G^{-1})

The data in Table (3) indicate significant differences between the sorbitol acid treatments. Treatments S_2 and S1 were significantly superior, yielding the highest levels of 3.260 and 3.097 mg.g^{-1} respectively, with no significant difference between them. Treatment S0, on the other hand, recorded the lowest level of 2.603 mg.g^{-1}. This reflects the positive role of sorbitol acid in improving the efficiency of the plant's metabolic system and increasing the green pigment content.

Regarding the effect of water stress, the results showed significant differences between the levels, with level W_0

significantly outperforming, recording the highest rate of 3.246 mg.g⁻¹, while the chlorophyll content decreased with increasing stress intensity, with W_1 and W_2 recording rates of 2.911 and 2.803 mg.g⁻¹ respectively, without significant differences between them. This indicates the negative effect of water stress in reducing chlorophyll content as a result of damage to chloroplasts or inhibition of its synthesis.

Regarding the interaction between sorbitol and water stress, the table data indicates that the interaction between S_2 and W_2 yielded the highest significant value of 3.333 mg/g⁻¹, which did not differ significantly from other treatments such as S_2 with W_0 and S_1 with W_0, which shared the same letter. On the other hand, the S0.W2 interaction had the lowest significant value of 2.230 mg/g.1. This indicates that the application of sorbitol at 40 mg L.1 has reduced the harmful effect of water stress on the high chlorophyll content of the plants, perhaps by its action in the regulation of osmotic potential and in the protection of cell membranes and enzyme systems involved in photosynthesis.

Table 4: Influence of sorbitol treatment and water.stress duration on foliar chlorophyll content (mg/g).

Sorbitol Rate	W0	W1	W2	Mean (Sorbitol)
S0	3.183ab	2.396c	2.230c	2.603b
S1	3.253a	2.870b	3.170ab	3.097a
S2	3.303a	3.143ab	3.333a	3.260a
Mean (Water Stress)	3.246a	2.911b	2.803b	

Values within the table designated by matching letters exhibit no statistically significant variation, as assessed by Duncan's multiple range test at the 0.05 probability level.

3.1.4. Effect of Sorbitol and Water Stress on Anthocyanin Content (Mg.100g⁻¹):

Results presented in Table (4) show that the differences between the sorbitol acids are statistically significant. The highest rate (65.345 mg/100g.1) was obtained for treatment S2 followed by S1 (54.812 mg/100g.1) and the lowest rate (45.354 mg/100g.1) was obtained for S0. This indicates the role of sorbitol acid as a catalyst for enhancing anthocyanin pigment accumulation**.**

In the case of water stress, the results indicated that there were significant differences between the water stress levels; the highest level was 68.847 mg/100g.1 in W2, the lowest level was 38.094 mg/100g.1 in W0 and the intermediate level was 58.570 mg/100g.1 in W1. This is a response of the plant under water stress and is a defense mechanism with production of secondary compounds (anthocyanins)**.**

Concerning the interaction between sorbitol and water stress, the data in the table shows that the S2 x W2 interaction had the highest significant value of 80.000 mg/100g.1 and the lowest significant value of 32.473 mg/100g.1 was the S0 x W0 interaction. Moreover, some treatments did not show significant differences with respect to letter combinations. Taken together these results indicated that as the level of water stress increased, there was a positive effect of sorbitol, especially 40 mg/L.1, on the accumulation of anthocyanins, which may be linked to its ability to regulate oxidative stress and to enhance the metabolic pathways responsible for the synthesis of these pigments.

Table 5: Effect of sorbitol and water stress on anthocyanin content (mg/100g)

Sorbitol Rate	W0	W1	W2	Mean (Sorbitol)
S0	32.473i	48.383f	55.206e	45.354c
S1	34.686h	58.413d	71.336b	54.812b
S2	47.123g	68.913c	80.000a	65.345a
Mean (Water Stress)	38.094c	58.570b	68.847a	

The values in the table with the same letters do not have significant differences between them according to Duncan's test with a probability of 0.05.

3.1.5. Effect of Sorbitol And Water Stress As A Function Of Phenol Content (Mg.G Wet Weight⁻¹)

The data in Table (5) regarding phenolic content (mg.g wet weight⁻¹) indicate significant differences between the sorbitol acid treatments. Treatments S_2 and S_1 were significantly superior, yielding the highest levels of 4.400 and 4.384 mg.g wet weight⁻¹ respectively, with no significant difference between them. Treatment S_0 recorded the lowest level of 3.697 mg.g wet weight⁻¹. This reflects the positive role of sorbitol in stimulating the accumulation of phenolic compounds.

Regarding the effect of water stress, the results showed clear and significant differences between the levels. Level W_2 showed the highest rate at 5.127 mg.g wet weight⁻¹, followed by level W1 at 4.328 mg.g wet weight⁻¹, while level W_0 showed the lowest rate at 3.025 mg.g wet weight⁻¹. This indicates that increased water stress intensity stimulated the accumulation of phenols as antioxidant defense compounds.

From the table data, it was observed that there was no significant interaction between S1 and W2 and S2 and W2 with the highest significant value of 5.463 and 5.276 mg/g wet weight respectively. On the other hand, the interaction of S0 and W0 gave the lowest significant value (2.673 mg/g wet weight). These findings indicate that sorbitol, especially in higher concentrations, promoted plant response to water stress by increasing the phenolics level, which is essential to alleviate oxidative damage to plant cells.

Table 6: Influence of sorbitol treatment and water.stress duration on phenolic content (mg/g fresh weight⁻¹).

Sorbitol Rate	W0	W1	W2	Mean (Sorbitol)
S0	2.673e	3.776c	4.643b	3.697b
S1	3.146d	4.590b	5.463a	4.384a
S2	3.256d	4.620b	5.276a	4.400a
Mean (Water Stress)	3.025c	4.328b	5.127a	

Values sharing common letter designations within the table demonstrate no significant statistical differences, as determined by Duncan's multiple range test at a 0.05 probability level.

3.1.6. Effect of Sorbitol and Water Stress on Flavonoid Characterization (Mg/G Wet Weight ^{1})

The data in Table (6) for the flavonoid content characteristic (mg.g wet weight^{-1}) indicate significant differences between the sorbitol acid treatments. Treatment S_2 was significantly superior, yielding the highest rate of 2.480 mg.g wet weight^{-1}, followed by treatment S_1 with a rate of 2.030 mg.g wet weight^{-1}, while treatment S_0 yielded the lowest rate of 1.547 mg.g wet weight^{-1}. This reflects the role of sorbitol in promoting the accumulation of flavonoid compounds.

Regarding the effect of water stress, the results showed significant differences between the levels, with level W_2 significantly outperforming, recording the highest rate of 2.400 mg.g wet weight^{-1}, followed by level W_1 with a rate of 2.233 mg.g wet weight^{-1}, while level W0 recorded the lowest rate of 1.424 mg.g wet weight^{-1}, indicating that increasing the intensity of water stress stimulated the production of flavonoids as a defense response.

As to interaction of sorbitol and water stress, it is found that there was no significant difference between the interaction of S2 and W1 (2.916 mg/g wet weight) and the interaction of S2 and W2 (2.776 mg/g wet weight) which gave the highest significant value. On the other hand, the combination of S0 and W0 showed the lowest significant value of 1.133 mg/g wet weight. Given the significance of flavonoids in the protection of plant cells and the resistance to oxidative stress, the results have shown that the addition of sorbitol, especially 40 mg/L, led to an increase in the content of these flavonoids in the water stress scenario.

Table 7: Effect of sorbitol and water stress on flavonoid characterization (mg/g wet weight.1)

Sorbitol Rate	W0	W1	W2	Mean (Sorbitol)
S0	1.133g	1.580ef	1.930d	1.547C
S1	1.393f	2.203c	2.493b	2.030B
S2	1.746de	2.916a	2.776a	2.480A
Mean (Water Stress)	1.424c	2.233b	2.400a	

Entries marked with identical letters in the table reflect no significant statistical divergence, as established by Duncan's multiple range test at the 0.05 probability threshold.

3.2. Discussion

The study results indicated that in most of the traits studied, the effects of sorbitol stress, water stress and their interactions were significant. The number of fruits, average fruit weight and chlorophyll content were increased with sorbitol particularly in 40 g/L.1 treatment compared to the control. This could be explained by the fact that sorbitol is a physiologically important osmotic regulating compound, which could help the plant maintain water balance inside the cells and minimize the impact of water stress. Also, it improves the photosynthetic efficiency and promotes the transport of photosynthetic products and carbohydrates to the fruits, thereby affecting plant growth and yield. Sorbitol also helps to preserve cell membranes and chloroplasts from oxidative damage from stress, resulting in higher levels of chlorophyll and higher efficiency of important plant life processes. The findings align with those of Kocaman (2024) [6], who found that the use of sorbitol in strawberry plants boosted their physiological response to stress and led to higher levels of chlorophyll and concentrations of phenolic compounds and flavonoids.

The reduction in fruit number, weight and chlorophyll content under irrigation intervals is explained by the water stress impact, which reduced the water and nutrient uptake, turgidity of the cells, cell division and elongation, reduced photosynthetic efficiency, and the damage to chloroplasts by increasing oxidative stress. This has a negative effect on the growth and productivity of plants. Under water stress conditions, Ripoll *et al.* (2014) [12] reported that water.deficiency causes physiological disturbances, reduced photosynthetic efficiency and decreased fruit growth in horticultural plants including strawberry, as it disrupts vital processes related to growth and production.

The results also revealed that the concentration of anthocyanin, phenol and flavonoids were significantly higher with increases in water stress and sorbitol application. The explanation of that is that these compounds are secondary compounds, whose production is related to antioxidant activity and plants under stress conditions have less damage due to the production of these compounds. Another possibility for improved synthesis of these compounds is that sorbitol may have had a positive effect on osmotic regulation and/or metabolic pathways involved in phenolics. The results are in line with those of Aaby *et al.* (2005) [1] who have shown that strawberries are rich in phenolic compounds, and anthocyanins, which are enhanced by adverse environmental factors as a result of the activation of antioxidant defense systems. In the same way, Giampieri *et al.* (2012) pointed out that among the most relevant bioactive compounds of strawberries are phenols, flavonoids and anthocyanins, whose accumulation has been related to an increase in the resistance of plants to different environmental stresses.

4. Conclusion

The study revealed that sorbitol, especially at 40 g/L^{-1}, promoted the growth and productivity of strawberry by increasing the number of strawberries, the average weight of strawberries and the content of chlorophyll. The plant's productive and physiological traits were decreased due to water stress, and phenolic compounds, flavonoids and anthocyanins were accumulated as a mechanism of defense against oxidative stress. The results also suggest that sorbitol is effective in reducing the impact of water stress and improving plant water stress resistance.

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