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The Role of Sorbitol in Reducing Water Stress on Strawberry Plants

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Abstract. This study was conducted in unheated protected greenhouses at the department of Horticulture and Landscape, College of Agriculture, Tikrit University, Iraq, during 2023–2024seasons. The aim was to study the role of sorbitol as a protective agent in reducing the effect of water stress on the growth and yield of the variety Ruby Gem Fragaria x ananassa . The experiment was carried out as a factorial experiment according to a randomized complete block design with three replicates. It included two factors: sorbitol concentrations (0, 20, and 40 g/L^{-1}) and water stress periods of 3, 5, and 7 days. The traits studied included number of fruits, weight of fruit, and chlorophyll content of leaves. Traits of anthocyanin, phenol, and flavonoid content of the fruits. 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} (S2) 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.

Keywords: sorbitol, strawberry, water stress, antioxidant compounds, chlorophyll

INTRODUCTION

The strawberry plant is a perennial herbaceous plant belonging to the order Rosales, family Rosaceae, genus Fragaria, species ananasa [1]. It is classified as a perennial plant that is adapted to a wide range of climatic conditions as it grows wild in its native habitat in North America [2]. Global strawberry production in 2012 reached 4,516,810 million tons, compared to 4,178,125 million tons in 2009, with a cultivated area of 241,109 hectares [3]. The reason for the increase in global consumption of strawberries is due to their antibacterial and antifungal properties, as they contain vegetables and phenols. The fruits are also characterized by their good nutritional value and their beneficial flavor, as they contain many substances and nutrients such as fats, carbohydrates, proteins, magnesium, calcium, phosphorus, potassium, copper, zinc, and vitamin C [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 [5]. 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 [6].

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 [7]. 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 [8]. Water stress also causes a decrease in plant height and the formation of undesirable and deformed fruit [9].

MATERIALS AND METHODS

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.

RESULTS AND DISCUSSION

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 (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

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.

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:

a. Without addition, denoted as S_0.
b. 20 g/L^{-1} S_1.
c. 40 g/L^{-1} S_2.
2. The second factor is the application of water stresses through irrigation periods, as follows:
a. Water every 3 days W_1.
b. Water every 5 days W_2.
c. Water every 7 days W_3.

The characteristics studied:

Average number of fruits (fruit.plant-^{1}):

Data were collected from the start of the harvest until the last harvest for the five plants in each experimental unit, and then the average number of fruits per plant was calculated as follows:

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

Average fruit weight (g):

The total weight of the fruits of the five marked plants was calculated and then divided by the total number of fruits to obtain the average fruit weight, as follows:

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

Leaf chlorophyll content (mg/100g soft weight $^{-1}$):

Chlorophyll was extracted from fresh leaf samples as described by Arnon [10], using 80% acetone. The optical density of the extract was then read at wavelengths of 645 and 663 nm to estimate the amount of chlorophyll (mg L^{-1}), according to the following equation:

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

Anthocyanin pigment (mg/100g $^{-1}$ soft weight):

The anthocyanin pigment content of the fruits was measured as reported by Ranganna [11].

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. [12] 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.

Estimation of flavonoids in mg/g $^{-1}$:

The total flavonoid content was estimated using the method of Ordoñez et al. [13] 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.

Effect of sorbitol, water stress and the interaction between them on the average number of fruits (fruit/plant-1):

The data in Table (2) 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. Effect of sorbitol, water stress and their interaction on the fruit count trait ($fruit.plant^{-1}$)

Rate	water stress			sorbitol
	W_2	W_1	W_0	
9.096 b	7.440 c	9.416 b	10.433 a	S_0
9.734 a	9.383 b	9.446 b	10.373 a	S_1
10.050 a	9.466 b	10.183 a	10.500 a	S_2
	8.763 c	9.682 b	10.435 a	Rate

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.

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

The data in Table (3) 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):

Rate	water stress			sorbitol
	W_2	W_1	W_0	
17.661 b	16.416 d	18.190 B	18.376 ab	S_0
18.133 a	17.460 c	18.450 Ab	18.490 ab	S_1
18.273 a	18.016 b	17.946 Bc	18.856 a	S_2
	17.297 c	18.195 B	18.574 a	Rate

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.

Effect of sorbitol and water stress on leaf chlorophyll content ($mg.g^{-1}$):

The data in Table (4) 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 lowest significant value of 2.230 mg/g^{-1} was seen in the interaction between S0 and W2. It indicates that the use of 40mg/L-1 concentration of sorbitol reduced the negative effect of water stress, and kept chlorophyll concentration high, which may be due to its osmotic effect and its protective action on the cell membranes and enzyme system responsible for photosynthesis.

Table 4. Effect of sorbitol and water stress on chlorophyll content in leaves (mg/g):

Rate	water stress			sorbitol
	W_2	**W_1**	**W_0**	
2.603 b	2.230 c	2.396 c	3.183 ab	**S_0**
3.097 a	3.170 ab	2.870 b	3.253 a	**S_1**
3.260 a	3.333 a	3.143 ab	3.303 a	**S_2**
	2.803 b	2.911 b	3.246 a	**Rate**

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.

Anthocyanin content (mg.100g-1) of response to sorbitol and water stress:

The figures in Table (5) show that there are significant differences between the sorbitol acid treatments. Treatment S2 gave the best result with 65.345 mg/100g^{-1} followed by treatment S1 with 54.812 mg/100g^{-1} and treatment S0 with 45.354 mg/100g^{-1}. This indicates that Sorbitol acid can be used as a catalyst to boost anthocyanin pigment production.

About the effect of the water stress the results indicated significant differences between the levels; level W2 exhibited the highest rate (68.847 mg/100g-1), followed by level W1 (58.570 mg/100g-1), and level W0 had the lowest rate (38.094 mg/100g-1). This is due to the plant's reaction to water stress by increasing the production of secondary compounds like anthocyanins as a defense mechanism.

As far as the interaction between sorbitol and water stress, the table data shows that the highest significant value is 80,000 mg/100g^{-1} for the interaction between S2 and W2 and the lowest significant value is 32,473 mg/100g^{-1} for the interaction between S0 and W0. In addition, there were no significant differences between treatments based on letter combinations. These findings indicate that with the intensity of water stress, the use of sorbitol at higher concentrations (40 mg/L^{-1}) led to the increase of anthocyanin accumulation which may be attributed to its antioxidant activity and activation of metabolic pathways involved in anthocyanin synthesis.

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

Rate	water stress			sorbitol
	W_2	W_1	W_0	
45.354 c	55.206 e	48.383 f	32.473 i	S_0
54.812 b	71.336 b	58.413 d	34.686 h	S_1
65.345 a	80.000 a	68.913 c	47.123 g	S_2
	68.847 a	58.570 b	38.094 c	Rate

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.

Effect of sorbitol and water stress as a function of phenol content (mg.g wet weight^{-1})

The data in Table (6) 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 means that with the more intense water stress, phenols accumulated as antioxidants defense compounds.

As to the effect of sorbitol and water stress, the data in the tables shows that the interaction between S1 and W2 and S2 and W2 were the highest with the significant values being 5.463 and 5.276 mg/g wet weight respectively; this difference was not significant. However, the lowest significant value of 2.673 mg/g wet weight was obtained for the interaction between S0 and W0. The results indicated that the sorbitol application, especially at high concentrations, helped the plants to better withstand water stress by increasing the accumulation of polyphenols, which are important in mitigating oxidative stress and maintaining cellular integrity.

Table 6. Effect of sorbitol and water stress on the phenol content profile (mg/g wet weight^{-1})

Rate	water stress			sorbitol
	W_2	W_1	W_0	
3.697 b	4.643 b	3.776 c	2.673 e	S_0
4.384 a	5.463 a	4.590 b	3.146 d	S_1
4.400 a	5.276 a	4.620 b	3.256 d	S_2
	5.127 a	4.328 b	3.025 c	Rate

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.

Effect of sorbitol and water stress on flavonoid characterization (mg/g wet weight $^{-1}$):

The data in Table 7. 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.

The table data showed that the interaction between sorbitol and water stress was the highest significant with 2.916 and 2.776 mg/g wet weight, in the case of S2 and W1 and S2 and W2, respectively, with no significant difference between them. On the other hand, the lowest significant value was recorded for S0 and W0, which is 1.133 mg/g wet weight. The results show that under water stress, the supplementation of sorbitol, especially at 40mg/l, resulted in an increased amount of flavonoids, which are important for the ability to withstand oxidative stress and protect plant cells.

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

Rate	**water stress**			**sorbitol**
	W_2	**W_1**	**W_0**	
1.547 C	1.930 d	1.580 ef	1.133 g	**S_0**
2.030 B	2.493 b	2.203 c	1.393 f	**S_1**
2.480 A	2.776 a	2.916 a	1.746 de	**S_2**
	2.400 a	2.233 b	1.424 c	**Rate**

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.

Study results indicated that sorbitol and water stress along with their interactions had significant effects on most of the studied traits. The number of fruits, average fruit weight, and chlorophyll content were higher in sorbitol treated plants than in the control treatment especially at the concentration of 40 g/L^{-1}. This could be explained by the role of sorbitol as an osmotically regulating compound, contributing to the plant's water balance in the intracellular space and minimizing adverse effects of water stress. Additionally, it improves photosynthetic efficiency and the translocation of photosynthetic products and carbohydrates to fruits which positively affect plant growth and yield. Sorbitol also helps to stabilize cell membrane and chloroplasts from oxidative stress and thus preserve high levels of chlorophyll and enhance the efficiency of essential processes in the plant. The outcomes obtained are in line with the findings of Kocaman [14], who showed that chlorophyll content increased and phenolic compounds and flavonoids were higher after strawberry plant treatment with sorbitol due to plant's physiological response to stress.

Lower number, weight and chlorophyll content in the fruits with increasing irrigation intervals are explained as due to water stress which causes reduced water and nutrient uptake, lowering of cell turgor pressure, inhibition of cell division and elongation, decreased photosynthetic efficiency and oxidative stress of the chloroplasts. This has an adverse effect on plant development and production. Deprivation of water causes physiological disturbances, reduced photosynthetic efficiency and reduced fruit growth in horticultural crops, such as strawberries, as a consequence of the disruption of important processes involved in growth and production, as reported by Ripoll et al. [15].

In addition, the results revealed that the anthocyanin, phenol and flavonoid concentrations were significantly higher at increasing water stress levels and in the presence of sorbitol. This can be attributed to the fact that these compounds are secondary compounds that have antioxidant activity, wherein plants produce more of these secondary antioxidants as a defense response to minimise the free radical accumulation under stress conditions. The improved synthesis of these compounds could also have been due to sorbitol's effect on regulating osmosis and stimulating metabolic pathways related to phenolic compounds. The results are in line with Aaby et al. [16], who found that strawberries are naturally rich in phenolics and anthocyanins which are stimulated during unfavorable environmental conditions by the stimulation of antioxidant defence mechanisms. In the same way, Giampieri et al. [17] reported that among the bioactive compounds found in strawberries it is possible to highlight the presence of phenols, flavonoids and anthocyanins, and the accumulation of these substances is linked to increased resistance of the strawberry plants to different environmental stresses.

CONCLUSION

The study showed that sorbitol had a positive effect on strawberry growth and productivity which depended on its concentration, the highest being at 40 g/L^{-1}, resulting in higher fruit production, average fruit weight, and chlorophyll content. Water stress was found to decrease the productive and physiologic attributes, whereas accumulation of phenolic compounds, flavonoid and anthocyanins was increased as a defense mechanism against oxidative stress. These results show the efficacy of sorbitol in reducing the impact of water stress and improve the ability of the plant to adapt to water stress conditions.

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