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Inducing Salinity Tolerance in Strawberry (*Fragaria* x *ananassa*) by Proline and Glycine Betaine

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Abstract: Salinity is a major abiotic stress factor that affects agricultural productivity worldwide, including strawberry cultivation. Implementing effective strategies to manage soil salinity is crucial for optimizing strawberry productivity. So, a research trial was conducted to evaluate the effects of external applications of proline (0, 75, 100, 150 ml L^{-1}, as main factor) and glycine betaine GB (0,150, 200, 250 mg L^{-1}, as sub main factor) on the performance, oxidation of fruit tissues, quantitative and qualitative yield of strawberry (*Fragaria x ananassa*) grown on saline soil having EC value of 7.5 dsm^{-1} via a split-plot experimental design across the successive seasons of 2021/2022 and 2022/2023. The findings showed that the growth performance parameters (*e.g.*, plant height, number of leaves), quantitative and qualitative yield indicators (*e.g.*, fruit weight, total yield, anthocyanin pigment, catalase enzyme CAT) of strawberry exhibited an increasing trend as the application rates of proline or GB increased. The highest values were observed when proline was applied at a rate of 150 ml L^{-1} in combination with GB at 250 mg L^{-1}. Regarding the malondialdehyde (MDA) as an oxidative stress indicator, the control treatment (without proline or GB) exhibited the highest levels of MDA. Conversely, the combined treatment of proline (150 ml L^{-1}) and GB (250 mg L^{-1}) demonstrated the lowest levels of MDA. Generally, it can be concluded that the soil salinity adversely affected strawberry growth, yield, and oxidative stress indicator. However, the external application of proline and GB proved to be beneficial in mitigating the salinity harmful effect.

Key words: Strawberry, proline, glycine betaine, yield and fruit quality.

INTRODUCTION

Strawberry (*Fragaria x ananassa*) hold great significance in the Egyptian market **(Abd-Elgawad, 2019)** contributing to the country's agricultural sector and economy **(Malhat *et al.*, 2020).** The Egyptian market recognizes the importance of strawberries as a high-value crop, providing a lucrative opportunity for farmers and producers **(Abd-El-Kareem *et al.*, 2022).** Salinity is a major abiotic stress factor that affects agricultural productivity worldwide **(Ghazi *et al.*, 2021)** including strawberry cultivation **(Zahedi *et al.*, 2020). El-Agrodi *et al.* (2016)**

exhibited that when exposed to high salt levels in the soil, strawberry plants experience osmotic stress and ion toxicity, as this can lead to nutrient imbalances **(El-Hadidi *et al.*, 2020)** reduction of water uptake, ion accumulation **(Soleymanzadeh *et al.*, 2020),** oxidative stress and finally reduced growth and yield (**Arıkan *et al.*, 2020**). Strawberries are relatively sensitive to salinity, thus exploring salt stress responses in strawberries is interesting **(Larson, 2018).** Implementing effective strategies to manage soil salinity is crucial for optimizing strawberry productivity **(Shamsabad *et al.*, 2022).** To combat the negative effects of salt affected soil, researchers have been exploring various strategies for enhancing the tolerance of strawberry plants to these harsh circumstances. One such approach involves the use of osmoprotectants, including proline and glycine betaine. Proline is an amino acid that acts as a compatible solute, aiding in the maintenance of cell turgor and stability under stressful environments **(Ibrahim *et al*., 2023).** Glycine betaine (GB), a quaternary ammonium compound, also functions as an osmoprotectant, assisting plants in osmoregulation and stress adaptation **(Annunziata *et al*., 2019).** Under stressful environmental conditions, GB has been observed to accumulate in the cytoplasm of various plants, acting as an osmolyte. The foliar application of GB has a stabilizing and protective effect on enzymes and membranes when strawberry plants are grown on salt-affected soil as mentioned by **Alaei and Mahna (2023)**.

These osmoprotectants have shown promising potential in mitigating the harmful effects of soil salinity on strawberry plants **(Heuer, 2003 and Ntanos *et al.*, 2021).** By applying proline and glycine betaine externally, their presence within the plant tissues can help regulate water balance **(Gerdakaneh *et al*., 2010; Aras and EşİtkEN, 2013; Kahlaoui *et al.*, 2018 and Nada, 2020),** reduce oxidative stress, and enhance overall plant performance **(Adak, 2019; Annunziata *et al.*, 2019; Alfosea-Simón *et al.*, 2020 and Mugwanya *et al.*, 2023).** Understanding the role of these osmoprotectants in improving salinity tolerance can provide valuable insights for farmers and researchers in effectively managing strawberry production in salt-affected soils.

Therefore, the main objective of this research work is to evaluate the effects of proline and glycine betaine on the performance, oxidative stress indicator, quantitative and qualitative yield of strawberries (*Fragaria x ananassa*) grown on salt-affected soil. By investigating the potential benefits of these osmoprotectants, this study aims to contribute to the development of sustainable strategies for enhancing strawberry cultivation in the presence of soil salinity, ensuring the continued success and profitability of this important crop in the Egyptian market.

MATERIALS AND METHODS

A research trial was conducted to evaluate the effects of external applications of proline (0, 75, 100, 150 ml L^{-1}, as main factor) and glycine betaine (0,150, 200, 250 mg $L^{-1,}$ as sub main factor) on the performance, oxidative stress, quantitative and qualitative yield of strawberry (*Fragaria x ananassa*) grown on saline soil with EC value of 7.5 dsm^{-1}.

Experimental site

A field experiment was executed during two successive seasons of 2021/2022 and 2022/2023 at a privately-owned farm situated in Seen Elbaharya village, Badr district, Buhaira governorate, Egypt.

Soil sampling

Prior to implementing the experiment, soil sample was collected (depth of 0-30 cm) then analyzed using the stander methods, as their characteristics are presented in Table 1.

Table (1). Characters of the initial soil (average of both seasons) depending on Sparks *et al.* (2020) and Dane and Topp (2020)

Parameters	Values
Particle size distribution (%)	
Clay	50.00
Silt	29.35
Sand	20.65
Textural class is clay	
Organic matter, %	1.39
EC dSm^{-1}(soil paste extract)	7.50
pH (soil suspension, 1: 2.5)	7.85
N, $mgKg^{-1}$	48.5
P, $mgKg^{-1}$	8.94
K, $mgKg^{-1}$	210.3

Studied substances

Proline and glycine betaine were purchased from Egyptian commercial market, then the studied rates were prepared.

Strawberry seedlings

Fresh seedlings of the Festival F1 hybrid strawberry (*Fragaria X ananassa Duch*.) variety were acquired from Techno Green Farms, located in New El-Salhia. The strawberry plants were transplanted on 15th October during both seasons. Prior to transplantation, all seedlings were carefully chosen based on their crown diameter, ensuring that each seedling had a crown diameter exceeding 0.5 cm.

Experimental setup

This trial was held out in a split plot design with three replicates, as the proline and glycine betaine at various studied rates were sprayed five times at 50, 65, 80, 95 and 110 after transplanting. Compost was added to the studied soil at a rate of 20 m^3 fed^{-1} three months before strawberry cultivation. In addition, the soil was sterilized before transplanting through solar sterilization. Calcium superphosphate fertilizer (15% P_2O_5) was added at a rate of 150 kg fed^{-1} then the soil was well- plowed and disked. Then, planting lines were planned, and then the lines were surveyed and divided into terraces with a width of 120 cm.

The experimental unit area was 14.4 m^2 (comprising three beds that were 1.6 m wide and 3.0 m long). Each bed accommodated four rows of transplants, resulting in a total of 140 plants per plot. For irrigation, each bed was equipped with two dripper lines spaced 25 cm apart, with a flow rate of 4 l/h. Before planting, the fresh strawberry transplants were immersed in a disinfectant solution (Rhizolex solution) at a concentration of 3.0 g per liter for a duration of 30 minutes. Following the disinfection process, the transplants were immediately planted, maintaining a spacing of 25 cm between each other on both sides of the dripper lines. Sprinkler irrigation was employed for the initial two weeks after transplantation, and subsequently, drip irrigation was utilized until the end of the growing season. In the 5th week after transplanting, the runners were removed and defoliation took place, followed by mulching the beds with 60-micron brown-silvery plastic mulch at the end of that week, which corresponded to 35 days from the transplanting date. In the phase of vegetative growth, every m^3 of irrigation water was supplemented with 500 cm3 of compound fertilizer containing 10% nitrogen (N), 2% phosphorus (P), 6% potassium (K), and microelements. During the flowering stage, the same amount of compound fertilizer, consisting of 10% nitrogen (N), 4% phosphorus (P), 8% potassium (K), and microelements, was added to each cubic meter of irrigation water. In the fruiting stage, 500 cm3 of compound fertilizer with 8% nitrogen (N), 2% phosphorus (P), 10% potassium (K), and microelements was incorporated into every m^3 of irrigation water.

Measurements

Growth performance traits

Samples of five plants were collected from the central three rows of each experimental plot to assess plant height (cm), foliage fresh weight (g $plant^{-1}$), secondary crown number $plant^{-1}$, number of leaves $plant^{-1}$, leaf area (cm^2 $plant^{-1}$), and leaf dry matter (%) after 120 days of transplanting in both seasons. Additionally, the levels of photosynthetic pigments were measured, including chlorophyll which measured using SPAD and carotene content (mg g^{-1}) which was analyzed as described by **Picazo *et al.* (2013).**

Yield and its components

Strawberry fruits were harvested when they reached a size suitable for the market. They were then weighed and counted to determine the yield (marketable and unmarketable, ton ha^{-1}) and average fruit weight (g). The cumulative yield from December to March was considered as the early yield, while the total yield was measured by recording the weights of all harvested fruits from each plot until 1st June. Also, fruit firmness (g/ cm^{-2}) and fruit dry matter (%) were measured.

Fruit quality

During the first week of April, using a random sample of ten fruits harvested, the following quality parameters were determined.

- Total soluble solids (TSS %, by a hand refractometer), total sugars % in dry matter), acidity (%) and vitamin C (VC, mg $100g^{-1}$, using titrimetric estimation with 2,6 dichloro phenol dye solution) were determined according to **AOAC (2000)**. To determine the titratable acidity, a random sample of 100g of fresh fruits was taken. The acidity was measured as grams of citric acid per 100g of juice. Anthocyanin (mg $100g^{-1}$) was measured as described by **Crecente-Campo *et al*. (2012).**

- Malondialdehyde (MDA, µmol. g^{-1} F.W, as an oxidative stress indicator) and catalase (CAT, unit.g^{-1}.min^{-1}, as an antioxidant enzyme) were measured by using spectrophotometric method as described by **Mendes *et al.* (2009) and Alici and Arabaci (2016),** respectively.

Statistical analysis

The data analysis was carried out using CoStat version 6.303(1998 -2004), as the statistical technique outlined in the methodology reported by **Gomez and Gomez (1984).** The least significant difference (LSD) test was at a significance level of 5%.

RESULTS

Growth criteria and photosynthetic pigments

Tables 2 and 3 illustrates the effects of different rates of proline and glycine betaine on vegetative growth characteristics of strawberries, including plant height (cm), foliage fresh weight (g $plant^{-1}$), secondary crown number $plant^{-1}$, number of leaves $plant^{-1}$, leaf area (cm^2 $plant^{-1}$), and leaf dry matter (%) after 120 days of transplanting during seasons of 2021/2022 and 2022/2023.On the other hand, Table 4 demonstrates the effects of the tested treatments on the levels of photosynthetic pigments, including chlorophyll (SPAD value) and carotene content (mg g^{-1}) after 120 days of transplanting in both seasons (2021/2022 and 2022/2023). The findings showed that the growth performance parameters and leaves photosynthetic pigments of strawberries exhibited an increasing trend as the application rates of proline or GB increased as individual effects for each alone. The lowest values of all aforementioned traits were achieved in the absence of both proline and GB. The highest values of all aforementioned traits were observed when proline was applied at a rate of 150 ml L^{-1} in combination with GB at 250 mg L^{-1}.

Table (2). Effect of different concentrations of proline and glycine betaine on plant height, fresh weight of foliage and number of secondary crown /plant after 120 days of transplanting of strawberry plant during 2021/2022 and 2022/2023 seasons

Treatments		Plant height (cm)		Fresh weight of foliage (g/plant)		Number of secondary crown /plant	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
		Effect of proline amino acid concentrations					
Control (without)		22.42	23.13	58.60	59.89	5.50	6.33
Proline (75 ml L^{-1})		22.98	23.69	61.71	62.95	5.58	6.83
Proline (100 ml L^{-1})		23.26	23.98	63.43	64.70	6.25	7.17
Proline (150 ml L^{-1})		23.41	24.14	64.19	65.52	6.50	7.42
LSD at 5%		**0.18**	**0.20**	**0.85**	**0.75**	**0.77**	**0.67**
		Effect of glycine betaine (GB) concentrations					
Control (without)		21.97	22.69	56.24	57.44	5.17	6.00
GB (150 mg L^{-1})		22.97	23.67	61.64	62.90	5.50	6.75
GB (200 mg L^{-1})		23.45	24.18	64.41	65.79	6.50	7.42
GB (250 mg L^{-1})		23.67	24.40	65.63	66.94	6.67	7.58
LSD at 5%		**0.30**	**0.26**	**0.67**	**0.66**	**0.77**	**0.78**
proline level	**GB level**	**Effect of interaction**					
Control	**Control**	21.66	22.34	54.32	55.43	5.00	5.67
	150 mg L^{-1}	22.50	23.25	59.11	60.24	5.67	6.33
	200 mg L^{-1}	22.69	23.40	60.00	61.44	5.67	6.67
	250 mg L^{-1}	22.81	23.52	60.99	62.46	5.67	6.67
75 mg L^{-1}	**Control**	21.87	22.66	55.70	56.96	5.00	6.00
	150 mg L^{-1}	22.95	23.60	61.44	62.77	4.00	6.67
	200 mg L^{-1}	23.45	24.20	64.31	65.61	6.67	7.33
	250 mg L^{-1}	23.62	24.31	65.38	66.47	6.67	7.33
100 mg L^{-1}	**Control**	22.11	22.82	56.95	58.12	5.33	6.00
	150 mg L^{-1}	23.12	23.82	62.63	63.89	6.00	7.00
	200 mg L^{-1}	23.77	24.48	66.35	67.76	6.67	7.67
	250 mg L^{-1}	24.05	24.82	67.79	69.02	7.00	8.00
150 mg L^{-1}	**Control**	22.24	22.93	58.00	59.26	5.33	6.33
	150 mg L^{-1}	23.30	24.02	63.36	64.69	6.33	7.00
	200 mg L^{-1}	23.90	24.64	67.00	68.33	7.00	8.00
	250 mg L^{-1}	24.18	24.97	68.38	69.81	7.33	8.33
LSD at 5%		**0.60**	**0.52**	**1.35**	**1.31**	**1.55**	**1.56**

Table (3). Effects of different concentrations of proline and glycine betaine on number of leaves/ plant, Leaves area and dry matter of leaves after 120 days of transplanting of strawberry plant during 2021/2022 and 2022/2023 seasons

Treatments		Number of leaves/ plant		Leaves area (cm^2 / plant)		Dry matter of leaves (%)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
		Effect of proline amino acid concentrations					
Control (without)		32.25	33.00	439.92	445.92	28.48	29.61
Proline (75 ml L^{-1})		34.25	35.17	472.58	478.08	29.03	30.34
Proline (100 ml L^{-1})		35.33	37.58	488.92	495.25	29.41	30.62
Proline (150 ml L^{-1})		36.17	38.58	496.83	503.92	29.58	30.77
LSD at 5%		**0.57**	**1.28**	**6.89**	**7.05**	**0.17**	**0.45**
		Effect of glycine betaine (GB) concentrations					
Control (without)		30.92	30.17	417.42	422.58	28.01	29.16
GB (150 mg L^{-1})		34.08	36.17	472.75	479.58	29.01	30.25
GB (200 mg L^{-1})		35.83	38.00	497.75	504.00	29.62	30.85
GB (250 mg L^{-1})		37.17	40.00	510.33	517.00	29.86	31.06
LSD at 5%		**0.96**	**1.45**	**12.43**	**11.13**	**0.14**	**0.73**
proline level	**GB level**	**Effect of interaction**					
Control	**Control**	29.00	28.33	399.67	403.33	27.68	28.86
	150 mg L^{-1}	33.00	33.00	444.67	452.00	28.54	29.66
	200 mg L^{-1}	33.33	35.00	453.33	459.67	28.75	29.89
	250 mg L^{-1}	33.67	35.67	462.00	468.67	28.95	30.02
75 mg L^{-1}	**Control**	31.00	29.00	414.67	421.00	27.94	29.17
	150 mg L^{-1}	34.00	36.33	474.67	480.33	28.75	30.19
	200 mg L^{-1}	35.67	35.67	495.00	500.33	29.63	30.96
	250 mg L^{-1}	36.33	39.67	506.00	510.67	29.81	31.02
100 mg L^{-1}	**Control**	31.33	31.33	423.33	428.33	28.14	29.28
	150 mg L^{-1}	34.33	37.00	482.67	491.00	29.26	30.40
	200 mg L^{-1}	37.00	40.33	517.67	523.00	29.97	31.21
	250 mg L^{-1}	38.67	41.67	532.00	538.67	30.27	31.58
150 mg L^{-1}	**Control**	32.33	32.00	432.00	437.67	28.28	29.35
	150 mg L^{-1}	35.00	38.33	489.00	495.00	29.50	30.74
	200 mg L^{-1}	37.33	41.00	525.00	533.00	30.13	31.35
	250 mg L^{-1}	40.00	43.00	541.33	550.00	30.40	31.63
LSD at 5%		**1.92**	**2.89**	**24.86**	**22.25**	**0.27**	**1.45**

Table (4). Effects of different concentrations of proline and glycine betaine on photosynthetic pigments in leaves of strawberry after 120 days of transplanting during 2021/2022 and 2022/2023 seasons

Treatments		Chlorophyll SPAD		Carotene mg/g dry weight	
		1st season	2nd season	1st season	2nd season
		Effect of proline amino acid concentrations			
Control (without)		44.34	45.02	0.244	0.248
Proline (75 ml L^{-1})		45.25	46.08	0.259	0.264
Proline (100 ml L^{-1})		45.75	46.44	0.267	0.273
Proline (150 ml L^{-1})		45.96	46.66	0.270	0.275
LSD at 5%		**0.64**	**0.26**	**0.002**	**0.004**
		Effect of glycine betaine (GB) concentrations			
Control (without)		43.73	44.38	0.233	0.238
GB (150 mg L^{-1})		45.22	45.89	0.259	0.263
GB (200 mg L^{-1})		46.01	46.83	0.272	0.277
GB (250 mg L^{-1})		46.33	47.10	0.276	0.282
LSD at 5%		**0.57**	**0.14**	**0.003**	**0.002**
proline level	**GB level**	**Effect of interaction**			
Control	**Control**	43.36	44.05	0.225	0.227
	150 mg L^{-1}	44.47	45.19	0.248	0.253
	200 mg L^{-1}	44.62	45.37	0.250	0.255
	250 mg L^{-1}	44.89	45.48	0.254	0.259
75 mg L^{-1}	**Control**	43.67	44.31	0.233	0.238
	150 mg L^{-1}	45.11	45.82	0.257	0.262
	200 mg L^{-1}	45.98	47.01	0.272	0.275
	250 mg L^{-1}	46.25	47.20	0.276	0.281
100 mg L^{-1}	**Control**	43.85	44.44	0.236	0.241
	150 mg L^{-1}	45.52	46.17	0.264	0.269
	200 mg L^{-1}	46.60	47.41	0.282	0.288
	250 mg L^{-1}	47.02	47.75	0.287	0.293
150 mg L^{-1}	**Control**	44.05	44.72	0.240	0.245
	150 mg L^{-1}	45.79	46.40	0.267	0.270
	200 mg L^{-1}	46.84	47.53	0.285	0.290
	250 mg L^{-1}	47.16	47.98	0.287	0.296
LSD at 5%		**1.14**	**0.29**	**0.006**	**0.005**

Fruit yield and quality

Tables 5 and 6 presents the impact of different rates of proline and glycine betaine on the yield characteristics of strawberries. These characteristics include average fruit weight (g), fruit firmness (g cm^{-2}), fruit dry matter (%), early and total yield (marketable and unmarketable, ton ha^{-1}) during the seasons of 2021/2022-2022/2023. While, Tables 7 and 8 demonstrates the effects of the studied treatments on various quality traits, such as TSS (%), total sugars (%), acidity (%), vitamin C (VC, mg $100g^{-1}$), anthocyanin (mg $100g^{-1}$), Malondialdehyde (MDA µmol g^{-1} F.W), and catalase (CAT, unit.g^{-1}.min^{-1}) in both seasons (2021/2022-2022/2023). The data analysis revealed that all the treatments examined in the study had a significant impact on the mentioned traits.

Table (5). Effects of different concentrations of proline and glycine betaine on fruit characteristics of strawberry plant during 2021/2022 and 2022/2023 seasons

Treatments		Average fruit weight (g)		Fruit firmness (g/ cm^2)		Dry matter of fruit (%)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
		Effect of proline amino acid concentrations					
Control (without)		20.29	20.58	328.83	332.83	7.93	8.07
Proline (75 ml L^{-1})		20.99	21.35	342.75	347.00	8.22	8.41
Proline (100 ml L^{-1})		21.34	21.67	349.67	354.08	8.36	8.55
Proline (150 ml L^{-1})		21.49	21.81	352.67	357.25	8.44	8.64
LSD at 5%		**0.27**	**0.28**	**5.21**	**2.87**	**0.16**	**0.24**
		Effect of glycine betaine (GB) concentrations					
Control (without)		19.86	20.14	317.33	320.67	7.67	7.84
GB (150 mg L^{-1})		20.92	21.23	343.50	348.25	8.21	8.43
GB (200 mg L^{-1})		21.54	21.91	354.33	358.92	8.47	8.66
GB (250 mg L^{-1})		21.79	22.13	358.75	363.33	8.61	8.76
LSD at 5%		**0.22**	**0.21**	**8.98**	**6.76**	**0.23**	**0.17**
proline level	**GB level**	**Effect of interaction**					
Control	**Control**	19.64	19.93	306.33	309.33	7.48	7.65
	150 mg L^{-1}	20.31	20.67	332.00	335.67	7.94	8.14
	200 mg L^{-1}	20.48	20.77	335.67	340.33	8.01	8.19
	250 mg L^{-1}	20.73	20.97	341.33	346.00	8.29	8.31
75 mg L^{-1}	**Control**	19.76	20.07	316.00	318.33	7.66	7.83
	150 mg L^{-1}	20.90	21.20	344.00	349.00	8.18	8.41
	200 mg L^{-1}	21.56	22.01	353.33	359.00	8.48	8.65
	250 mg L^{-1}	21.72	22.11	357.67	361.67	8.56	8.76
100 mg L^{-1}	**Control**	19.95	20.19	322.00	326.33	7.72	7.89
	150 mg L^{-1}	21.16	21.46	346.33	350.67	8.31	8.53
	200 mg L^{-1}	21.99	22.36	364.00	367.67	8.67	8.85
	250 mg L^{-1}	22.27	22.65	366.33	371.67	8.75	8.95
150 mg L^{-1}	**Control**	20.08	20.35	325.00	328.67	7.81	7.99
	150 mg L^{-1}	21.32	21.60	351.67	357.67	8.40	8.63
	200 mg L^{-1}	22.14	22.49	364.33	368.67	8.72	8.93
	250 mg L^{-1}	22.42	22.78	369.67	374.00	8.82	9.02
LSD at 5%		**0.45**	**0.42**	**17.95**	**13.53**	**0.46**	**0.34**

Regarding all above mentioned traits, except MDA and acidity, the values increased as the rate of proline or glycine betaine increased under both individual and interaction statistical analysis. In other words, the combined treatment of proline (150 ml L^{-1}) and (250 mg L^{-1}) caused the maximum values of average fruit weight (g), fruit firmness (g cm^{-2}), fruit dry matter (%), early and total yield (marketable and unmarketable, ton ha^{-1}), TDS (%), total sugars (%), vitamin C (VC, mg $100g^{-1}$), anthocyanin (mg $100g^{-1}$) and catalase (CAT, unit.g^{-1}.min^{-1}).

Table (6). Effects of different concentrations of proline and glycine betaine on yield characteristics of strawberry plant during 2021/2022 and 2022/2023 seasons

Treatments		Early yield (ton ha^{-1})				Total yield (ton ha^{-1})			
		Marketable		Unmarketable		Marketable		Unmarketable	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
		Effect of proline amino acid concentrations							
Control (without)		12.10	12.36	1.71	1.65	51.67	52.60	2.33	2.44
Proline (75 ml L^{-1})		13.31	13.58	1.64	1.63	54.62	55.52	1.99	2.18
Proline (100 ml L^{-1})		13.94	14.23	1.64	1.59	56.21	57.24	1.77	1.61
Proline (150 ml L^{-1})		14.23	14.50	1.66	1.61	56.89	57.76	1.70	1.83
LSD at 5%		**0.23**	**0.18**	**0.01**	**N.S**	**0.30**	**0.72**	**0.03**	**0.68**
		Effect of glycine betaine (GB) concentrations							
Control (without)		11.20	11.42	1.77	1.72	49.54	50.36	2.66	2.83
GB (150 mg L^{-1})		13.32	13.61	1.68	1.61	54.74	55.73	1.87	1.86
GB (200 mg L^{-1})		14.35	14.64	1.61	1.58	57.06	58.03	1.71	1.80
GB (250 mg L^{-1})		14.71	15.00	1.58	1.56	58.04	59.01	1.54	1.57
LSD at 5%		**0.13**	**0.15**	**0.02**	**N.S**	**0.17**	**0.52**	**0.02**	**0.76**
Proline level	**GB level**	**Effect of interaction**							
Control	**Control**	10.58	10.79	1.59	1.58	47.81	48.94	3.02	3.24
	150 mg L^{-1}	12.31	12.61	1.83	1.80	52.11	53.01	2.19	2.26
	200 mg L^{-1}	12.60	12.88	1.74	1.62	52.92	53.76	2.17	2.29
	250 mg L^{-1}	12.92	13.16	1.67	1.59	53.83	54.69	1.95	1.97
75 mg L^{-1}	**Control**	11.07	11.27	1.79	1.77	49.11	49.61	2.81	3.57
	150 mg L^{-1}	13.19	13.45	1.59	1.55	54.57	55.53	2.19	2.13
	200 mg L^{-1}	14.31	14.62	1.67	1.70	57.02	58.14	1.52	1.55
	250 mg L^{-1}	14.65	14.98	1.52	1.50	57.77	58.79	1.43	1.46
100 mg L^{-1}	**Control**	11.44	11.69	1.76	1.66	50.15	50.96	2.47	2.20
	150 mg L^{-1}	13.72	14.07	1.64	1.51	55.84	56.95	1.57	1.45
	200 mg L^{-1}	15.10	15.38	1.57	1.59	58.88	59.94	1.55	1.55
	250 mg L^{-1}	15.53	15.79	1.57	1.60	59.97	61.12	1.48	1.24
150 mg L^{-1}	**Control**	11.72	11.93	1.93	1.88	51.09	51.92	2.33	2.31
	150 mg L^{-1}	14.05	14.33	1.67	1.59	56.43	57.43	1.55	1.59
	200 mg L^{-1}	15.37	15.67	1.47	1.43	59.44	60.25	1.62	1.82
	250 mg L^{-1}	15.76	16.07	1.57	1.53	60.61	61.44	1.31	1.62
LSD at 5%		**0.26**	**0.30**	**0.04**	**0.04**	**0.34**	**1.04**	**0.04**	**1.53**

Concerning the malondialdehyde (MDA) as an oxidative stress indicator and acidity, the control treatment (without proline or GB) exhibited the highest levels of MDA and acidity, as their values decreased as the rate of proline or glycine betaine increased under both individual and interaction statistical analysis. In other words, the combined treatment of proline (150 ml L^{-1}) and GB (250 mg L^{-1}) demonstrated the lowest values of MDA and acidity.

Table (7). Effects of different concentrations of proline and glycine betaine on total soluble solids (TSS), total sugars and acidity in fruits of strawberry plant during 2021/2022 and 2022/2023 seasons

Treatments		TSS %		Total sugar %		Acidity %	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
		Effect of proline amino acid concentrations					
Control (without)		7.51	7.67	5.99	6.11	0.731	0.742
Proline (75 ml L^{-1})		7.96	8.03	6.21	6.34	0.706	0.721
Proline (100 ml L^{-1})		8.19	8.35	6.35	6.48	0.694	0.706
Proline (150 ml L^{-1})		8.29	8.47	6.40	6.54	0.685	0.702
LSD at 5%		**0.09**	**0.21**	**0.09**	**0.05**	**0.009**	**0.009**
		Effect of glycine betaine (GB) concentrations					
Control (without)		7.25	7.39	5.82	5.98	0.742	0.755
GB (150 mg L^{-1})		7.94	8.03	6.22	6.34	0.721	0.725
GB (200 mg L^{-1})		8.32	8.49	6.42	6.53	0.706	0.699
GB (250 mg L^{-1})		8.44	8.60	6.50	6.61	0.702	0.691
LSD at 5%		**0.09**	**0.12**	**0.06**	**0.07**	**0.008**	**0.007**
proline level	**GB level**	**Effect of interaction**					
Control	**Control**	7.00	7.12	5.65	5.80	0.750	0.765
	150 mg L^{-1}	7.56	7.74	6.04	6.20	0.730	0.747
	200 mg L^{-1}	7.70	7.84	6.09	6.17	0.725	0.731
	250 mg L^{-1}	7.80	7.95	6.18	6.28	0.719	0.726
75 mg L^{-1}	**Control**	7.19	7.34	5.77	5.94	0.746	0.761
	150 mg L^{-1}	7.88	7.70	6.21	6.32	0.711	0.725
	200 mg L^{-1}	8.32	8.50	6.41	6.50	0.687	0.702
	250 mg L^{-1}	8.44	8.57	6.45	6.58	0.682	0.698
100 mg L^{-1}	**Control**	7.31	7.46	5.89	6.03	0.739	0.744
	150 mg L^{-1}	8.11	8.26	6.28	6.40	0.705	0.719
	200 mg L^{-1}	8.61	8.79	6.58	6.71	0.672	0.685
	250 mg L^{-1}	8.73	8.88	6.66	6.78	0.662	0.675
150 mg L^{-1}	**Control**	7.48	7.63	5.97	6.14	0.732	0.752
	150 mg L^{-1}	8.23	8.40	6.33	6.45	0.687	0.710
	200 mg L^{-1}	8.66	8.84	6.62	6.75	0.666	0.679
	250 mg L^{-1}	8.80	8.99	6.69	6.82	0.653	0.665
LSD at 5%		**0.17**	**0.24**	**0.11**	**0.14**	**0.016**	**0.013**

Table (8). Effects of different concentrations of proline and glycine betaine on Vitamin C, Anthocyanin, Malondialdehyde and Catalase in fruits of strawberry plant during 2021/2022 and 2022/2023 seasons

Treatments		Vitamin C (mg/100g fresh weight)		Anthocyanin (mg /100g fresh weight)		Malondialdehyde (MAD) (µmol/g fresh weight)		Catalase (CAT) (unit /g/min.)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
		Effect of proline amino acid concentrations							
Control (without)		53.25	53.61	54.93	56.67	8.20	8.22	3.70	3.77
Proline (75 ml L^{-1})		54.39	55.09	56.51	58.21	7.42	7.50	4.13	4.18
Proline (100 ml L^{-1})		55.09	56.03	57.25	59.01	6.95	7.02	4.34	4.40
Proline (150 ml L^{-1})		55.41	56.33	57.60	59.36	6.71	6.81	4.45	4.49
LSD at 5%		**0.73**	**0.30**	**0.93**	**0.96**	**0.14**	**0.08**	**0.05**	**0.11**
		Effect of glycine betaine (GB) concentrations							
Control (without)		52.25	52.62	53.88	55.59	8.56	8.67	3.44	3.47
GB (150 mg L^{-1})		54.35	55.09	56.52	58.24	7.47	7.56	4.13	4.18
GB (200 mg L^{-1})		55.51	56.47	57.75	59.53	6.75	6.85	4.47	4.53
GB (250 mg L^{-1})		56.02	56.89	58.14	59.89	6.50	6.47	4.59	4.64
LSD at 5%		**0.66**	**0.17**	**0.68**	**0.71**	**0.21**	**0.09**	**0.09**	**0.09**
Proline level	**GB level**	**Effect of interaction**							
Control	**Control**	52.59	51.75	52.96	54.60	8.75	8.85	3.17	3.20
	150 mg L^{-1}	53.22	53.86	55.21	57.08	8.04	8.14	3.77	3.85
	200 mg L^{-1}	53.42	54.18	55.62	57.39	7.92	8.03	3.90	3.98
	250 mg L^{-1}	53.76	54.66	55.95	57.63	8.07	7.86	3.98	4.04
75 mg L^{-1}	**Control**	51.78	52.44	53.71	55.53	8.61	8.70	3.39	3.42
	150 mg L^{-1}	54.48	55.19	56.35	57.92	7.57	7.66	4.08	4.14
	200 mg L^{-1}	55.47	56.20	57.84	59.63	6.82	6.91	4.46	4.50
	250 mg L^{-1}	55.81	56.55	58.12	59.74	6.66	6.72	4.59	4.64
100 mg L^{-1}	**Control**	52.13	53.01	54.22	56.01	8.50	8.63	3.50	3.55
	150 mg L^{-1}	54.69	55.41	57.04	58.69	7.21	7.30	4.26	4.32
	200 mg L^{-1}	56.41	57.62	58.64	60.40	6.24	6.33	4.74	4.79
	250 mg L^{-1}	57.12	58.08	59.09	60.92	5.83	5.80	4.87	4.94
150 mg L^{-1}	**Control**	52.49	53.29	54.63	56.22	8.36	8.48	3.69	3.73
	150 mg L^{-1}	55.02	55.90	57.48	59.27	7.04	7.14	4.38	4.42
	200 mg L^{-1}	56.75	57.86	58.91	60.68	6.02	6.12	4.80	4.85
	250 mg L^{-1}	57.40	58.28	59.39	61.29	5.43	5.51	4.92	4.95
LSD at 5%		**1.32**	**0.35**	**1.36**	**1.41**	**0.42**	**0.18**	**0.20**	**0.17**

DISCUSSION

The results of this investigation demonstrated the potential of proline in combination with glycine betaine (GB) for enhancing salinity stress tolerance in strawberry plants. Proline played a vital role in mitigating the detrimental effects of salinity on strawberry leaves, primarily by serving as an osmoprotectant and a compatible solute. In strawberries grown on salt-affected soil, proline may act as an osmoprotectant, antioxidant, compatible solute and regulator of ion homeostasis **(Ibrahim *et al.*, 2023).** Through its accumulation in leaves and these various functions, proline helps maintain cellular water balance, stabilize proteins and cellular structures, scavenge reactive oxygen species (ROS) and regulate ion levels. These combined mechanisms contribute to the enhanced tolerance of leaves to stress under saline conditions. Similarly, glycine betaine (GB) may also function as an osmoprotectant in strawberry plants, aiding in osmoregulation and stress adaptation **(Annunziata *et al.*, 2019).** When exposed to salinity, GB has the ability to accumulate in the cytoplasm of strawberry plants, acting as an osmolyte.

The observed increase in catalase values in strawberry fruits with higher rates of proline or glycine betaine can be attributed to their potential role as enzymatic antioxidants, as proline and glycine betaine possess strong antioxidant characteristics and can act as ROS scavengers in strawberry plant cells. When applied individually, proline and glycine betaine enhance the activity of catalase, which is involved in the breakdown of H_2O_2 and O_2. As the rates of proline or glycine betaine increase, more of these antioxidants are available in the plant cells, leading to higher catalase activity. Moreover, when proline and glycine betaine are combined at specific concentrations, such as proline (150 ml L^{-1}) and glycine betaine (250 mg L^{-1}) in this case, they may synergistically enhance the antioxidant capacity of the strawberry plant. This synergistic impact could further boost catalase activity, resulting in the observed maximum values. Overall, the increased catalase activity with higher rates of proline or glycine betaine, both individually and in combination, suggests their role in promoting the enzymatic antioxidant defense system of the strawberry plants **(Annunziata *et al.*, 2019 and Ibrahim *et al.*, 2023).**

The results of this research trial provide valuable insights into the effectiveness of both proline and glycine betaine in mitigating the harmful impacts of soil salinity on strawberry cultivation. The findings demonstrate improvements in growth performance and yield indicators when these osmoprotectants were applied externally at various rates. The observed increase in growth performance parameters (*e.g.*, plant height, number of leaves), as well as quantitative and qualitative yield indicators (*e.g.*, fruit weight, total yield, anthocyanin pigment) due to the combined treatment of proline (150 ml L^{-1}) and GB (250 mg L^{-1}), can be attributed to the role of proline and glycine betaine in maintaining cell turgor and stability.it is known that, under salinity stress, strawberry plants experience water deficits due to increased osmotic potential in the soil. By applying both proline and glycine betaine, the strawberry plants were better equipped to regulate water balance, resulting in improved growth performance, thus improved quantitative and qualitative yield. In other words, the salinity stress of the studied soil negatively affects strawberry plant metabolism, nutrient uptake, and reproductive processes, leading to reduced total yield. The exogenous application of proline and glycine betaine helped to alleviate these adverse effects, promoting higher fruit yield and quality.

The observed decrease in acidity levels with increasing rates of proline or glycine betaine can be explained by their influence on the physiological processes and metabolism of the strawberry plants **(Bingöl *et al.*, 2020).** Acidity in fruits is primarily attributed to organic acids, such as citric acid and malic acid **(Yosefi *et al.*, 2020).** Proline and glycine betaine are known to have regulatory effects on various metabolic pathways, including those related to organic acid synthesis and degradation **(Bahmani *et al.*, 2022).** When the plants were treated with higher rates of proline or glycine betaine, it is possible that these substances influenced the activity of enzymes involved in organic acid metabolism **(Adak, 2019).** This could lead to a reduction in the production or accumulation of organic acids in the fruits, thereby resulting in decreased acidity levels **(Nada, 2020).** Moreover, the combined treatment of proline (150 ml L^{-1}) and glycine betaine (250 mg L^{-1}) may have exerted a synergistic effect on the metabolic pathways related to organic acid synthesis and degradation. This synergistic effect could have further suppressed the production or accumulation of organic acids, leading to the lowest acidity levels observed in the combined treatment.

The levels of malondialdehyde (MDA) serve as indicators of oxidative stress in the strawberry plants grown under salinity conditions. The studied salt-affected soil often led to increased production of reactive oxygen species (ROS) (**Singh *et al.*, 2014**), causing lipid peroxidation and cellular damage. In this study, the control treatment without proline or glycine betaine exhibited higher levels of MDA, indicating elevated oxidative stress. In contrast, the combined treatment of proline and glycine betaine demonstrated lower levels of MDA, suggesting reduced oxidative damage. Proline and glycine betaine act as antioxidants, scavenging ROS and protecting cellular structures from oxidative harm. These findings support previous studies **(Gerdakaneh *et al*., 2010; Aras and Eşİtken, 2013; Kahlaoui *et al*., 2018 and Nada, 2020)** that have demonstrated the positive effects of proline and glycine betaine in mitigating salinity-induced stress in various plant species. Finally, it can be said that the properties of both proline and glycine betaine help regulate ion transport, maintain osmotic balance and stabilize cell membranes as well as scavenge ROS (**Ntanos *et al*., 2021 and Alaei & Mahna, 2023**). These mechanisms collectively contribute to improved strawberry plant performance and salinity stress tolerance.

CONCLUSION

Based on the results, it can be concluded that the high salinity level of the studied soil adversely affected strawberry plant growth, yield, and oxidative stress indicators. However, the external application of proline and glycine betaine (GB) proved to be beneficial in mitigating the harmful effects of salinity. Increasing the application rates of proline and GB resulted in improved growth performance, quantitative and qualitative yield parameters, and reduced oxidative stress in strawberry plants. Notably, the combined treatment of proline (150 ml L^{-1}) and GB (250 mg L^{-1}) exhibited the most favorable outcomes across the studied parameters.

Finally, the following recommendations can be made: Strawberry growers should consider using external applications of proline and glycine betaine to alleviate the adverse effects of salt-affected soil on strawberry crops. The recommended rates of 150 ml L^{-1} for proline and 250 mg L^{-1} for glycine betaine have shown promising results in terms of growth, yield, and oxidative stress reduction. Additional studies should be conducted to investigate the long-term effects and sustainability of proline and glycine betaine applications on strawberry crops. Glycine betaine implementing these recommendations, strawberry growers can enhance their production of strawberries in salt-affected areas, mitigate the negative influences of soil salinity, ensure sustainable agricultural practices and ensure the continued success and profitability of this important crop in the Egyptian market.

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