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Research Article

Investigation of Foliar L-Glutamic Application on the Resistance to the Capacity of the SC2121 Tomato Variety (*Solanum lycopersicum* L.) to Long-Term Salinity Stress

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Abstract: Within the scope of this study, the effects of L-Glutamic acid (L-GLU: 250 mg L^{-1}, 500 mg L^{-1}) treatments on morphological and biochemical characteristics of SC2121 tomato variety under salt stress (50 mM, 100 mM, 200 mM NaCl) were compared. The morphological results obtained from leaves and fruits were found to peak at 500GLU, 50NaCl-500GLU, 250GLU, and 200NaCl-500GLU, whereas their lowest values were achieved with doses of 200NaCl, 200NaCl-250GLU, 100NaCl, and 100NaCl-500GLU Among the bioactive molecules, amino acid, and proline amounts increased in all the treatments, whereas total protein increased in 500GLU and 50NaCl- 250GLU, 50NaCl-500GLU. CAT activity increased in doses of 500GLU and 50 NaCl-250GLU, 50NaCl-500GLU, whereas POD and SOD activity decreased in high NaCl and 200NaCl+ 250GLU, 200NaCl-500GLU. Treatments caused an increase in MDA concentration, while NaCl (50-100 mM), GLU, and 100 NaCl-500GLU reduced the H_2O_2 concentration. In conclusion, 500GLU, 50NaCl-500GLU, 50NaCl-250GLU, 250GLU, and 200NaCl+500GLU stimulated the growth and development in the SC2121 tomato variety, as well as the leaf bioactive chemicals. However, 200NaCl-250GLU, 200NaCl, and 200NaCl-250GLU reduced the growth and development of the tomato and decreased the chemicals in the leaves. Given the results, it can be stated that yield and quality could be increased by making use of GLU treatments in tomato varieties under salt stress.

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1. Introduction

Salinity is one of the most progressive environmental factors suppressing the productivity of agricultural plants, excluding the salt-tolerant genotypes. Salinity problems can naturally develop in arid and semi-arid areas in regions with low precipitation or very uneven distribution and parameters such as excessive and unconscious irrigation in agricultural lands, the use of low-quality irrigation water, and high doses of fertilizers (Çelik et al., 2022; Kıpçak et al., 2019). It was researchers reported that approx. 20% of cultivated areas and 50% of irrigated areas worldwide are affected by salinity, 10 million hectares of land become unusable every year, and this amount will continue to increase. It was reported that data, approximately 6% of the world's land is threatened by salinity (Yılmaz et al., 2022). In Türkiye, 1.5 million hectares of land face salinity problems, and 60% of these areas are salty, 19.6% moderately saline, 0.4% moderately alkaline, and 12% mildly saline (Gursoy, 2022). Salinity influences plant morphologically, physiologically, and biochemically, and lead to serious losses in yield and quality (Tuncer, 2017; Can et al., 2021). It was proven that physiological reactions promoting growth and development such as photosynthesis, respiration, nitrogen and carbohydrate metabolism, soil-water-plant relations, and cell cycle are suppressed due to salinity stress (Sadak et al., 2014; Gursoy, 2022). In the studies aiming to solve the salinity problem, methods such as 1) reclamation of saline soils, 2) traditional breeding methods, modern molecular biology methods, and 3) selection and development of salinity-resistant species and varieties are primarily used (Turhan and Şeniz, 2010; Can et al., 2021). The first option is expensive and difficult, the second option is time-consuming, and the methods obtained cannot guarantee genetically modified products. Therefore, it seems that the most suitable way in agricultural lands having high salinity levels is the third option, which is the exogenous application of biosimultans such as amino acids, hormones, jasmonates, polyamines, and minerals to plants (Bahjat et al., 2022; Alp and Şensoy 2023). Researchers reported that NH_4^+ in the amino acid structure participated in the structure of organic compounds directly and with less energy and that exogenous amino acid applications were more advantageous in increasing plant yield and stimulating stress resistance (Çakır, 2017; Sun et al., 2019). They have a carboxylic acid, hydroxyl, sülfidril, and histidine group, an amino group, and a side chain, which arrest some elements such as Cl, and Na, and chelate some of them (Hildebrandt, 2018; Kuşvuran et al., 2019). Moreover, since they inhibit the synthesis of reactive radicals or stimulate the antioxidative defense systems, they play an important role in protecting plant cell structures against salinity stress effects (Noroozlo et al., 2019). Glutamate, as the main NH_4^+ donor, is metabolized to other amino acids such as proline, glycine, and arginine, and also plays an important role in chlorophyll biosynthesis, activation of plant hormones, cell division, and phytochelatin activities (Septiyana et al., 2019).

In the present study, it was aimed to reveal the effects of salt stress and foliar L-Glutamic acid applications on seedling growth, pomological measurements in fruit, and the amounts of some bioactive components in leaves in SC2121 tomato (*Solanum lycopersicum* L.) variety. As a result, determining the most suitable GLU doses, which minimize the effect of salt stress on the growth capacity of shoot/root, leaf, flower and fruit, and leaf phytochemicals, is important to recommend tomato cultivation in salt-stressed agricultural lands. This study is also of significant importance as it is the first to examine the effects of L-Glutamic acid on salt stress resistance of the SC2121 tomato genotype.

2. Material and Methods

This study was carried out in a garden in Elmadağı district of Ankara province between 17 April-28 July 2022. SC2121 tomato variety was used as research material. It is one of the most preferred varieties because it is early growing. It is suitable for field cultivation, suitable for table consumption, round and red in color, and thin-shelled (Omar Bohalima, 2017).

2.1. Description of Study Area

Elmadağı is a district that is 31km away from Ankara province, has a surface area of 647 $km^{2,}$ and an altitude of 1088 m. The district has been established between 39° 55' 19.6716" N and 33° 13' 34.5540" E coordinates and it is the 492nd largest district in Turkey. This region has a continental climate, in which winters are cold and harsh. As presented in Table 1, the temperature ranged between 11.25 and 23.46 ºC during the study period; the highest temperature was found to be 38.46 ºC in July and the lowest one was found to be 2.55 ºC in April. The average level of precipitation ranged between 14.10 and 51% and peaked in May (Table 1). The characteristics of the soil and water samples used are presented in Table 2. For the soil and water samples used, pH ranged between 6.88 and 7.02 and conductivity ranged between 0.58 and 142.2, whereas N content (%) was found to be 1.68-5.14, K to be 33.46-8850 mg kg^{-1}, P to be 116.44-6076 mg kg^{-1}, Fe to be 4.40-345 mg kg^{-1}, Zn to be -4.64 28.4 mg kg^{-1}, and n to be 1.17-87.8 mg kg^{-1}.

Table 1. Monthly average meteorological values of the study area

Months	Mean (°C)	Maximum (°C)	Minimum (°C)	Precipitation (mm)	Humidity (%)
April	11.25	25.12	2.55	42.20	56
May	16.14	35.22	12	51.30	53
June	20.18	30.16	12.95	35.20	51
July	23.46	38.46	15.85	14.10	50

Table 2. Properties of soil and water used in the experiment

	pH	Conductivity ($\mu S\ cm^{-1}$)	N	K	P	Fe	Zn	Mn
Soil	7.02	0.58	5.14	8600	5064	255	28.4	43.2
Manure	6.88	1.33	1.68	8859	6076	345	24.6	87.8
Water	7.82	142.2	-	33.46	116.44	4.4	4.64	1.17

2.1.1. Arrangement of experimental design of the study

First of all, soil mortar was prepared by mixing garden soil and barnyard manure 2:1. First of all, soil mortar was prepared by mixing garden soil and barnyard manure 2:1. Some of the soils were filled in viols containing 32 cells (35x35 diameter, 48 mm depth, 38 cc) and some in polyethylene seedling growing tubes (25x50 cm). Viols and pots 1- Control (0), 2- 50 mM NaCl (50NaCl), 3-100 mM NaCl (100NaCl), 4- 200 mM NaCl (200NaCL); 5- 250 mg/L L-Glutamic acid (250GLU), 6-500 mg/L L-Glutamic acid (500GLU); 7- 50 mM NaCl + 250 L-GLU (50NaCl-250GLU), 8- 50 mM NaCl + 500 L-GLU (50NaCl-500GLU), 9- 100 mM NaCl + 250 GLU (100NaCl-250G), 10-100 mM NaCl + 500 GLU (100NaCl-500G), 11- 200 mM NaCl + 250 GLU (200NaCl-250GLU) and 12- 200 mM NaCl + 500 GLU (200NaCl-500GLU) were classified and labeled as 12 groups. Some of the seedlings grown from the viols were used for morphological measurements and chemical analyzes on shoots, roots, and foliage. Some of them were transferred to seedling growing bags when they were at the 2-3 leaf stage and they waited for 2 weeks for their adaptation to the new environment before starting the applications. On the other hand, the measurements of the flower, fruit, branch, and shoot of these plants were carried out with seedlings developed in plastic bags. In the pot trials, a total of 9 pots were used with 3 replications for each group. NaCl applications ($NaCl_2$: Sodium chloride-CAS 7647-14-5) were made by giving NaCl solutions dissolved in bottled water (viol: 10 ml and bag: 350 ml) from the soil, whereas L-GLU (L-Glutamic acid: Sigma-Aldrich-CAS 616281) applications were carried out by sprinkling GLU doses dissolved in pure water on the lower and upper surfaces of the leaves with a sprayer. Applications to seedlings in the viols and tubes were made twice a week for 8 weeks. After the termination of the applications, the seedlings in the viols were harvested and used in morphological and chemical analyses. The number of leaves, the number of branches, root diameter, and fruit characteristics were determined in plants grown in plastic bags. The L-GLU concentration applied in the study was determined by a preliminary study. For the GLU concentration, it was selected by considering the germination capacity of the seeds which caused an average 50% increase, while the NaCl concentrations were determined by considering the germination capacity that increased by more than 50% and caused more than 50% inhibition (Sadak et al., 2015; Yılmaz et al., 2022).

2.1.2. Morphological measurements

After the treatments, the seedlings in vials were removed by paying attention to not damaging the roots, the soil particles on the roots were eliminated by using tap water, and then they were used in morphological measurements. Shoot length and root length were measured using a ruler. The dry and fresh weights of shoots and roots, and also the fruit weights were measured using a precision scale, root collar, and fruit length and fruit width were measured by a digital caliper. And, the numbers of leaves, branches, and flowers were determined by counting per plant. While 10 plants were used for each character measured for seedling and leaf development, the measurements of fruit characteristics were carried out with 10 randomly selected fruits.

2.1.3. Biochemical analysis

The chlorophylls, carotenoids, and xanthophylls were homogenated with ethanol and estimated according to the methods described by Kukric et al. (2012) and Chang et al. (2013). Total anthocyanin level measures following the methods described by Burgos et al. (2013).

Total free proline concentration was determined by following Bates et al. (1973) method. A 500 mg fresh leaf was homogenized with 10 mL sulfosalicylic acid and filtered. Then 2 mL of filtrate was mixed with 2 mL acid ninhydrin solution, 2 mL glacial acetic acid, and 4.0 mL of toluene in a test tube, the absorbance was taken at 520 nm. Whilst the protein content was determined according to the Bradford method (1976), total free amino acid to the Spies (1957).

MDA content was estimated following the original method of Lutts et al. (1996). H_2O_2 level was measured spectrophotometrically according to the method of Velikova et al. (2000).

The superoxide dismutase (SOD) activity was assayed by its ability to inhibit the photochemical reduction of nitrotetrazolium blue chloride (NBT) at 560 nm (Çakmak 1994). The catalase (CAT) activity was assayed by monitoring the degradation of H_2O_2 at 240 nm over 2 min against a supernatant-free blank (Bergmeyer, 1970). The peroxidase (POX) activity was based on the method described by Lee and Lin (1995). All chemical analyses were done with three replications.

2.1.4. Statistical analysis

One-way ANOVA (Analysis of variance) was applied for analyzing the differences in the growth parameters, fruit characteristics, and chemical constituents in the leaf samples of tomato seedlings. The statistical analysis was performed using the SPSS program (Version 11 for Windows). Following the results of ANOVAs, Tukey's honestly significant difference (HSD) test (α= 0.05) was used for significance.

3. Results and Discussion

3.1. Variation of growth parameters of seedling

Foliar application of GLU is one of the most up-to-date agricultural approaches to improving the growth and development, yield, and quality of agricultural species under abiotic stresses (Fardus et al., 2021; Engin and Gökbayrak, 2022). In the present study, the effects of GLU treatments on the growth parameters of SC2121 tomatoes subjected to salt stress are presented in Table 3. The highest values for shoot length and root length, shoot fresh weight and root fresh weight, shoot dry weight and root dry weight, and numbers of leaves and branches per plant were obtained using GLU doses, 50NaCl+ 250GLU, 50NaCl-500GLU, and 200NaCl-500GLU (Table 3). Moreover, shoot length and root length values increased in all the treatment groups in comparison to the control, and shoot fresh weight and root dry weight showed significant increases with GLU and NaCl-GLU, whereas root fresh weight increased significantly in 500GLU and NaCl-GLU doses (Table 3). On the other hand, among the parameters examined, whereas fresh shoot weight reached the minimum level with 200NaCl, root fresh weight with 250GLU and 200NaCl, dry root weight with 100NaCl and 200 NaCl, and the number of leaves per plant with 200NaCl (Table 3). The results found for the effects of NaCl and GLU applications on the shoo-root development and leaf numbers are in parallel with the literature. The negative effects of salt stress were reported for tomato (Turhan and Şeniz, 2010), for common beans (Kıpçak et al. 2019), and wheat (Yılmaz et al., 2022). However, many researchers also reported that the exogenous applications of amino acids were effective in recovering the plant from salt stress or reducing the negative effects (Jannesari et al., 2016; Alp and Şensoy, 2023). Similar to the present study, Sadak et al. (2015) examining broad beans and Souri et al. (2017) investigating tomato, squash, and bean plants grown in calcareous soils determined that exogenous amino acid treatments stimulated the shoot and root length; shoot fresh weight, root fresh weight, shoot dry weight, root dry weight; the number of leaves, leaf development, and yield. In other studies carried out on okra (Greenwell and Ruter, 2018) and lettuce (Noroozlo et al., 2019), it was found that glutamine, arginine, and glycine/glutamine applications stimulated the shoot and root development in those plants.

Table 3. Variation of the growth parameters of SC 2121 tomato seedlings under salinity (L-GLU: Glutamic acid)

Group	Doses	Shoot length (cm)	Root length (cm)	Shoot FW (g)	Shoot DW (g)	Root FW (g)	Root DW (g)	Leaves /per plant
Control	0	20.33±1.20b*	5.26±0.68c	3.43±0.18c	1.04±0.03b	1.11±010c	0.21±0.03c	29.9±2.25c
NaCl mM	50	23.11±2.34b	5.72±0.44c	3.90±0.65c	1.02±0.07b	1.10±0.14c	0.20±0.03c	32.90±3.14c
	100	21.77±1.17b	7.45±1.12b	3.36±0.26c	1.02±0.05b	1.19±0.08c	0.20±0.04c	29.80±1.82c
	200	18.18±1.07c	7.36±0.80b	3.04±0.17c	0.94±0.04b	0.99±0.07c	0.23±0.05c	24.70±1.40
L-GLU mg L^{-1}	250	25.52±1.77a	8.91±0.89a	5.09±1.16b	1.34±0.19a	0.95±0.06c	0.31±0.03b	38.7±2.84
	500	28.61±3.50a	9.76±1.40a	7.83±2.09a	1.30±0.20a	1.53±0.24a	0.33±0.04b	44.90±7.12b
NaCl/L-GLU	50/250	26.01±2.17a	7.73±0.49b	6.44±1.05a	1.18±0.09b	1.66±0.11b	0.57±0.08a	40.40±3.83b
	50/500	25.54±1.56a	6.66±0.83b	6.76±0.65a	1.38±0.14a	1.61±0.14b	0.43±0.06b	51.10±5.90a
	100/250	15.96±1.88d	7.66±0.70b	3.77±0.73c	0.63±0.10c	1.15±0.14c	0.29±0.03b	34.80±4.99c
	100/500	14.76±1.01d	7.74±0.82b	3.43±0.41c	0.38±0.02d	1.38±0.12b	0.27±0.03b	27.80±3.47c
	100/250	17.92±2.76c	7.77±1.20b	4.49±1.20b	0.82±0.19bc	1.61±0.26b	0.35±0.07b	29.86±4.12c
	200/500	24.36±4.52b	9.10±1.34a	7.74±2.81a	1.47±0.4a	2.81±0.86a	0.41±0.10b	36.90±6.1b1
F		3.617	1.929	2.151	3.404	3.172	4.141	2.195
P		<0.001	<0.043	<0.022	<0.001	<0001	<0.001	<0.020

*Means (±: n=3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $p \leq 0.05$.

In the present study, the decrease in shoot height in 100NaCl-250GLU, and 100NaCl-500GLU, and 200NaCl-250GLU in comparison to the control group were thought to be a response to salt-related water deficiency in the soil, reduced shoot length. Results found on the number of leaves per plant confirm this finding (Table 3). When compared to the control group, the treatments decreased the seedling root collar, number of branches per plant, and number of flowers per plant but resulted in an increase in the number of leaves per plant (Table 4). The number of leaves peaked at 100NaCl, 500GLU, and 50NaCl-50GLU. The number of branches and the fruit characteristics found in the present study were similar to those reported by Kavasoğlu and Ceyhan, (2018) for beans, Civelek and Yıldırım (2019) for tomatoes, and Septiyana et al. (2019) for okra. In another study, Omer et al. (2013) examining the chamomile plants grown under salty conditions reported that the plant height, the number of branches, and the number of flowers decreased under salty conditions but branch number and flower yield increased in response to the amino acid treatments. The decrease in the number of flowers was also reported by Nahed et al. (2007). Geshnizjanı and Khosh-Khui (2016) and Septiyana et al. (2019), differing from the present study, stated that amino acid treatment increased the number of flowers. However, Jannesari et al. (2016) emphasized that it stimulated the fruit yield under salty conditions but amino acid treatments had no significant effect. In the present study, glutamic acid stimulated the number of leaves and fruit development but had no effect on blooming. In the present study, glutamic acid stimulated the number of leaves and fruit development but had no effect on blooming. It was thought to be because of differences in the reproductive proliferation differences of species, highness of treatment (NaCl, GLU) doses, and duration.

Table 4. Variation of root, leaf, branch, flower and fruit characteristics of SC 2121 tomato seedlings under salinity (L-GLU: Glutamic acid)

Group	Doses	Root collar	Leaf number	Branch number	Flower number	Fruit Width (cm)	Fruit Length (cm)	Fruit FW
Control	0	5.30±0.30a*	217.0±28.57h	45.8±7.06a	23.0±2.30a	4.06±0.30b	3.72±0.37c	36.8±7.78f
NaCl mM	50	4.32±0.11b	272.8±20.32d	24.6±1.66f	14.2±1.02c	4.76±0.25b	3.82±0.16c	41.8±6.79e
	100	4.94±0.22a	447.8±59.97a	27.8±2.78c	17.0±2.85b	4.86±0.65b	3.94±0.34b	53.4±14.99d
	200	4.46±0.08b	242.4±15.84g	22.8±1.16e	12.4±0.75d	5.74±0.36a	4.84±0.35a	75.4±10.91a
L-GLU mg L^{-1}	250	4.44±0.06b	221.8±25.55h	26.2±2.58c	16.8±2.91b	5.16±0.50a	3.90±0.19b	61.6±21.90b
	500	4.80±0.20b	374.6±12.94b	24.0±1.82e	14.8±1.32c	4.96±0.55a	3.96±0.17b	53.8±9.31d
NaCl/L-GLU	50/250	4.94±0.17a	318.2±25.64c	29.8±4.88c	18.6±3.78b	4.04±0.12b	3.46±0.30d	33.6±3.43d
	50/500	4.64±0.10b	263.2±25.14e	24.2±1.07f	18.0±1.38b	5.46±0.46a	4.70±0.36a	76.8±19.02a
	100/250	4.20±0.10b	253.8±26.22f	23.2±2.46e	13.8±0.92c	4.72±0.12b	4.26±0.09b	57.6±3.43c
	100/500	4.38±0.10b	304.0±5.57c	24.8±2.38f	14.8±0.59c	4.12±0.19b	3.74±0.05c	32.0±3.10f
	100/250	4.42±0.06b	231.2±22.98g	23.0±2.45e	14.0±1.14c	3.84±0.14b	3.52±0.15d	34.4±1.69f
	200/500	4.64±0.19b	299.2±7.69c	33.0±1.87b	15.6±1.12c	4.24±0.33b	3.80±0.22c	39.6±9.64e
F		4.225	6.113	4.370	2.163	2.732	2.915	2.003
P		<0.001	<0.001	<0.001	0.033	<0.008	<0.005	<0.049

*Means (±: n=3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $p \leq 0.05$.

3.2. Effects of NaCl and GLU treatments on the bioactive chemical constituents of samples

Chlorophyll molecules are the major light-harvesting pigment, involved in the conversion of carbon dioxide in the air into energy-rich compounds, which are necessary to survive the life cycle of plants (Rudiger, 1997; Greenwell and Ruter, 2018). In the present study, the salt concentrations other than the dose of 200NaCl did not cause any decrease in chlorophyll content. On the other hand, the doses of 100NaCl-250GLU, 100NaCl-500GLU, and 200NaCl-250GLU significantly reduced the chlorophyll a and b and total chlorophyll contents in comparison to the control group (Table 5). The ratio of chlorophyll a to chlorophyll b decreased in all the treatment groups when compared to the control group, whereas total carotenoid content decreased in 100NaCl-250GLU, 100NaCl-500GLU, and 200NaCl-250GLU. The highest level of decrease in the chlorophyll molecules was observed in 100NaCl + 250GLU, 100NaCl-500GLU, whereas the highest level of stimulation was found in 50NaCl + 250GLU, 50NaCl-500GLU (Table 5). The increase in chlorophyll pigments in low (50 and 100 mM) NaCl doses suggests that the SC2121 variety was tolerant to low salt concentrations (Turfan, 2017; Çelik and Karakurt, 2022). However, the fact that the highest level was found in 50NaCl+250GLU, and 50NaCl-500GLU showed that the low level of glutamic acid application reduced the damage caused by salt stress (Sadak et al., 2015; Rivera et al., 2022). The parameter affected by the treatments the most was the ratio of chlorophyll a to chlorophyll b (Table 5). Chlorophyll a has a high level of sensitivity to stress conditions such as light, drought, and salt (Rudiger, 1997). Transformation of chlorophyll a to chlorophyll b in the SC2121 tomato variety might be an adaptive mechanism. According to the results, it was revealed that exogenous GLU applications were found to be positively effective at low salt (50NaCl) concentrations, and the highest NaCl.

Table 5. Variations of the amount of chlorophyll pigment and total carotenoid under salinity

Group	Doses	Chlorophyll a mg g^{-1}	Chlorophyll b mg g^{-1}	Total Chlorophyll mg g^{-1}	Chlorophyll a/ Chlorophyll b	Total carotenoid mg g^{-1}
Control	**0**	0.381±0.001d*	0.163±0.001g	0.544±0.002f	2.35±0.002a	7.36±0.01b
NaCl mM	**50**	0.399±0.001d	0.212±0.001e	0.611±0.001d	1.89±0.003c	7.20±0.01b
	100	0.446±0.002c	0.195±0.001f	0.640±0.001d	2.29±0.013a	7.68±0.01b
	200	0.274±0.001f	0.214±0.001e	0.488±0.001g	1.29±0.009e	5.39±0.01
L-GLU mg L^{-1}	**250**	0.532±0.001c	0.374±0.001c	0.905±0.001c	1.43±0.007d	9.38±0.01a
	500	0.499±0.002c	0.265±0.003d	0.764±0.0018d	1.88±0.030c	8.40±0.02b
NaCl/L-GLU	**50/250**	0.803±0.001a	0.481±0.002a	1.283±0.002a	1.67±0.005	10.89±0.01a
	50/500	0.773±0.002b	0.395±0.002b	1.167±0.004b	1.96±0.008b	10.58±0.01a
	100/250	0.184±0.001g	0.090±0.001h	0.273±0.001ı	2.05±0.014b	2.99±0.01d
	100/500	0.293±0.004e	0.143±0.001g	0.426±0.001h	1.89±0.003c	5.17±0.01c
	100/250	0.308±0.009e	0.149±0.001g	0.456±0.008g	2.07±0.076b	5.30±0.02c
	200/500	0.478±0.001c	0.223±0.001e	0.700±0.002d	2.14±0.006a	7.51±0.02b
F		4121.51	6664.93	12473.135	164.47	51519.996
P		<0.001	<0.001	<0.001	<0.001	<0.001

*Means (±: n=3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $p \leq 0.05$

Similarly, in studies aiming to decrease the damages of salt stress, it was observed that chlorophyll pigments decreased depending on genotype and salt concentration but exogenous bio-stimulant implementations caused an increase in the pigment content (Sadak et al., 2015; Fardus et al., 2021). The higher chlorophyll a, chlorophyll b, and total chlorophyll contents found in 250GLU, and 500GLU doses in the present study were related to the effectiveness of GLU in chlorophyll biosynthesis (An et al., 2019). Similar results were reported for lettuce (Noroozlo et al., 2019), and cucumber (Ikbal et al., 2021). The amount of xanthophyll was lowest in the control, conversely, it peaked in 200NaCl, 200NaCl-250GLU, and 200NaCl-500GLU doses (Figure 1A). This finding showed that this variety was tolerant to low salt concentrations but sensitive to high salt concentrations (Turhan and Şeniz, 2010; Fardus et al., 2021). Hence, Qiu and Lu (2003) carried out a study on *Atriplex centralasiatica*, a halophilic plant, and reported that carotenoids stimulated salt tolerance and xanthophyll increased the herbal resistance by protecting the light-harvesting pigment systems.

GLU treatments might have exhibited their positive effects on xanthophyll via the synthesis of required compounds and the activation of enzymes (Johnson et al., 2010; Cirillo et al., 2021). Anthocyanin concentration was low in 100NaCl-500GLU, 200NaCl-250GLU, and 200NaCl treatment groups but GLU increased in 50NaCl + 25GLU, and 50NaCl-500GLU (Figure 1B). Glutamic acid solely stimulated the anthocyanin accumulation but its effect on salt damage was observed in low NaCl+ GLU doses (Figure 1B). This might be because GLU doses activated the enzymes that were responsible for anthocyanin (Gould et al., 2002; Turfan and Turan, 2023).

Anthocyanin accumulation was reported to be stimulated by mechanical injury in pepper plant in a study by Gould et al. (2002), by high temperature in Arabidopsis in a study carried out by Shao et al. (2007), and tobacco in a study carried out by Cirillo et al. (2021) and it was also reported to protect the plants from stress damages. Amino acids and total soluble protein are important osmolytes playing a significant role in cells in osmosis, regulation of turgor reactions, and also, being metabolized and forming the precursors of many compounds (Wang et al., 2014). In the present study, NaCl, GLU, and NaCl-GLU applications caused an increase in total amino acid and proline content, but the total protein content increased only in 500GLU, 50NaCl + 250GLU, 50NaCl-500GLU (Figure 1C, Figure 1D, Figure 2 A). It was expected that amino acid and proline content would be high in GLU doses (Figure 1C, 1D). However, the fact that these molecules were at a high level in NaCl treatments might be because they catalyze total protein into amino acids and proline (Wang et al., 2014; Bahjat et al., 2022). Moreover, GLU treatment might have increased salt resistance by stimulating the proline synthesis pathway (Sun et al., 2019).

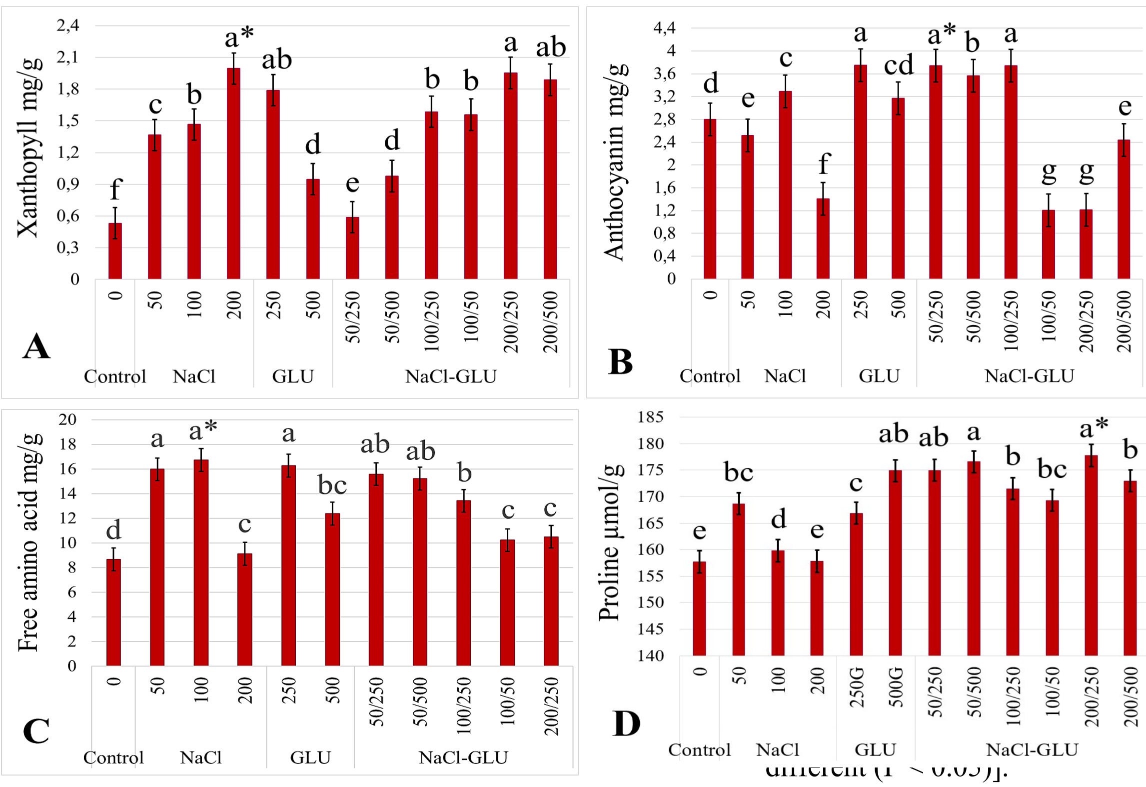

Figure 1. Variations of xanthophyll (1A), anthocyanine (1B), free amino acid (1C), and proline (1D) concentrations of SC2121 tomato seedling [(*: Means indicated with different letters within the same column are significantly different (P < 0.05)].

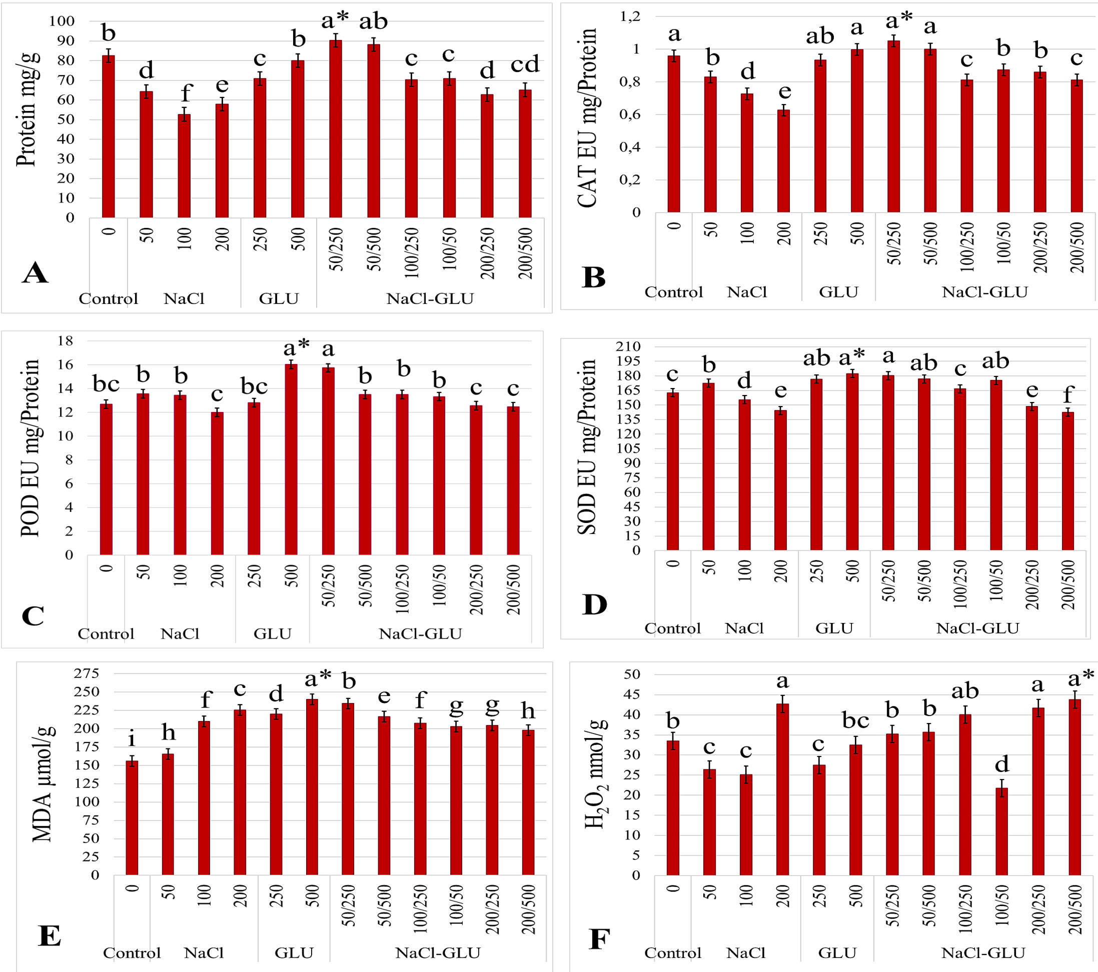

Figure 2. Variation of soluble protein (2A), CAT (2B), POD (2C) and SOD activities (2D), MDA (2E), and H_2O_2 concentrations of SC2121 tomato seedlings; [(*: Means indicated with different letters within same column are significantly different ($P < 0.05$)].

Many researchers reported that proline accumulation in cells increased in cases of water deficiency and salty conditions (Erdinc et al., 2018; Kıpçak et al., 2019). Besides them, proline might have induced the synthesis of nitrogenous compounds also by accelerating nitrate transportation (Bahjat et al., 2022). Similar to that study, Souri et al. (2017), Sun et al. (2019), and Turfan and Turan (2023) observed that GLU treatments caused increases in total amino acid and protein content in vegetative and reproductive organs. Similarly, Haghighia et al. (2020) also found that exogenous amino acid implementation given to squash seedlings significantly increased amino acid, protein, and proline content in comparison to the control group. Enzymatic molecules of defense mechanisms such as CAT, POD, and SOD suppress ROS production in plants under stress conditions or they scavenge these compounds from the cells (Ikbal et al., 2019; Yılmaz et al., 2022). In the present study, CAT activity was stimulated by 500GLU and 50NaCl +250GLU, 50NaCl-500GLU, and POD activity decreased in high NaCl and NaCl + GLU (Figure 2B, 2C). SOD activity was inhibited in 100NaCl, 200NaCl, 200NaCl + 25GLU, and 200NaCl-500GLU (Figure 2D). POD and CAT are the enzymes increasing the herbal resistance by reducing H_2O_2 into O_2 and H_2O (Figure 2E, 2F). Hence, in the present study, H_2O_2 content was found to be at a low level in high salt concentrations, in which POD activity was high. Moreover, the CAT, POD, and SOD activities were at the lowest level in the 200NaCl group and H_2O_2 content was high in this concentration (Figures 2B, 2C, 2D, 2F). Treatments stimulated CAT activity only in 500GLU and 50NaCl + 250GLU, and 50NaCl-500GLU (Figure 2B). In previous studies examining cucumber seedlings under Cd stress (Munawar et al., 2022) and lentil under salt stress (Fardus et al., 2021), it was reported that exogenous amino acid treatments stimulated the POD, SOD, and CAT activities. As in other organisms, plants also require oxygen (O_2) for an optimal life. However, during the metabolic reactions of cells and tissues, O_2 converts into toxic compounds and causes the accumulation of molecules known as reactive oxygen species (ROS) and triggering destructions or degenerations in structures of cell membranes, proteins, chlorophyll, and other organic molecules (Kıpçak et al., 2019; Çelik and Karakurt, 2022). Deformation in cell membrane structure results in an increased intracellular MDA concentration and might reach very high levels, especially under stress conditions (Kuşvuran et al., 2019; Gursoy, 2022). Similar to the present study, Ikbal et al. (2019) observed that MDA content decreased in leaves of cucumbers under salt stress but MDA concentration increased again when seedlings were given salt + GLU treatment. Researchers claimed that exogenous GLU treatment stimulated fatty acid denaturation under salty conditions and induced an increase in MDA content (Hildebrandt, 2018; Fardus et al., 2021).

Conclusion

In the study, the effects of L-GLU treatments on morphological and biochemical characteristics of the SC2121 tomato variety under salt stress were investigated. It has been revealed that at the 500GLU, 50NaCl-500GLU, 250GLU, and 200NaCl-500GLU the morphological values were the highest, but they were the lowest with 200NaCl, 200NaCL-250GLU, 100NaCl, and 100NaCl-500GLU. In addition, amino acid and proline amounts were higher in all the treatment groups, whereas total protein and CAT activity generally increased with 500GLU and 50NaCl+250GLU, 50NaCl-500GLU. POD and SOD activity stimulated by high NaCl and 200NaCl+ 250GLU, and 200NaCl-500GLU. MDA level enhanced with all applications, while the H_2O_2 concentration reduced with lower NaCl and GLU doses. Finally, it can be stated that GLU doses and 50NaCl+250GLU, and 50NaCl-500GLU increased the growth and development in the SC2121 tomato, as well as bioactive chemicals, therefore, yield and quality could be increased by making use of GLU treatments in tomato varieties under lower salt stress.

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